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SECTION MEETINGS

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| CLEVELAND | |
| Western Reserve University | January 10, 1941 |
| DISTRICT OF COLUMBIA | |
| U. S. Public Health Service | February 6, 1941 |
| ILLINOIS | |
| Northwestern University | January 21, 1941 |
| MINNESOTA | |
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| PACIFIC COAST | |
| University of California | February 5, 1941 |

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### 11939 P

## Cataracts in *Amblystoma tigrinum* Larvae Fed Experimental Diets.

ESTHER M. PATCH. (Introduced by W. J. Meek.)

*From the Department of Physiology, University of Wisconsin Medical School.*

Lens opacities in larvae of the tiger salamander were first observed by the author in members of an Illinois strain fed a semi-synthetic diet containing 45% of purified casein (repeated treatment with dilute alkali), 37% of powdered whole milk, cooked cornstarch, cod-liver oil, and bakers' yeast. Mature cortical cataracts developed in 40% of the animals living for 2 months or longer on this diet, while the eyes were normal in larvae of the same lot fed a diet of powdered beef muscle, cooked cornstarch, fat, minerals, and the vitamins. The disease did not appear in larvae fed the casein diet supplemented by cystine[1] (1.25% of the total protein of the diet), a result confirmed by repetition of the work with 2 sets of Wisconsin larvae. Cystine has also been found a preventative of cataracts[2, 3] produced in rats by casein diets.

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[1] Patch, E. M., *Science*, 1934, **79**, 57.

Cataracts were next observed in *Amblystoma tigrinum* larvae that had been fed for 3 months or more on synthetic rations of the separate proteins prepared from fresh muscle (extraction by an alkaline-buffered KCl solution and precipitation by graded acid dialysis). Seven rations were made like the earlier one of powdered whole muscle, each with a different muscle protein: beef myosin, beef globulin x, these 2 proteins and myogen from the white muscle of chicken, and the extraction residue (largely stroma protein) from both kinds of meat. All of these rations produced cataracts in larvae from both Tennessee and Wisconsin, with a total occurrence of 51%, while the disease did not appear in larvae of the same sources fed raw muscle or liver or synthetic diets of these tissues. The lens changes produced by the diets of the separate muscle proteins were like the changes caused by the diet of purified casein: rarely there was visible an early stage of radiating striae, which was followed by complete cortical disintegration and final disappearance of the shrunken lens into the vitreous chamber. Dietary supplementation with riboflavin[4] (1.25 $\gamma$ per gram of ration) did not prevent these cataracts. Twice the quantity of cystine which had been effective with the casein diet, was preventative with 2 of the muscle-protein diets, but not with 2 others. The latter may have needed a higher amount of free cystine; or possibly, during the preparation of the separate muscle proteins, some additional constituent of whole muscle necessary for lens health may have been lost.

Cortical cataracts with a very different history appeared in the carnivorous salamander larvae when they were fed synthetic diets of dried whole blood (beef or turtle), defibrinated blood, or hemoglobin. After 4 or 5 weeks on these rations, all of the larvae showed an easily visible black band of zoning in the outer cortical region of the lens and parallel to its surface. In 37% of the animals, whitening of the cortex followed, accompanied by a large fluid content within the lens capsule during a prolonged period of maturity. The lens zoning developed in larvae fed a diet of globin without heme, but not in those fed a diet of heme (3 times the amount in the hemoglobin diet) combined with powdered beef muscle instead of with globin. Also, no lens disturbance developed in larvae whose

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[2] Bourne, M. C., and Pyke, M. A., *Biochem. J.*, 1935, **29**, 1865.

[3] Yudkin, A. M., and Geer, H. A., *Arch. Ophth.*, 1940, **23**, 28.

[4] Day, P. L., Darby, W. J., and Cosgrove, K. W., *J. Nutrition*, 1938, **15**, 83.

sole source of protein was fibrin. Addition of riboflavin to the faulty blood diets did not prevent the cataracts, and cystine alone was not preventative when it made 7% of the dietary protein. There was, however, a definitely lower occurrence and intensity of the disease when both cystine (3.3%) and glutamic acid (15%) were added to the diet; and this preventative effect was still greater when glycine (3%) accompanied them. Incomplete prevention with the liberal supply of the three acids suggests inadequacy of some additional dietary component.

*Summary.* Two types of cortical cataract have been produced in salamander larvae by synthetic diets differing in their protein constituents. The first type, rarely detectable in the early stages and with a marked final shrinkage, was caused by a diet of purified casein and by diets of the separate muscle proteins. The second type, with an easily visible early zoning, large fluid content at maturity, and halted later changes, was caused by diets with high hemoglobin content. Prevention of the first type of cataract was attained by dietary supplementation with cystine, while prevention of the second type required all 3 components of glutathione and apparently some additional factor.

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## 11940

## Ferrous and Ferric Iron in Liver Extract.

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In recent years much attention has been paid to the relative efficiency of absorption and utilization of ferrous iron as compared with ferric iron in treatment of the iron deficiency anemias. It is not our purpose further to discuss this question, but rather to present the results of an investigation carried out on solutions of liver extract to which iron salts had been added.

In general, ferrous ions in solution are rapidly oxidized to the ferric state unless protected by some reducing agent. Borgen and Elvehjem[1] commented on the power of homogenized chicken liver extracts to reduce ferric iron. We had previously noted considerable reducing power in a simple aqueous liver extract. These facts sug-

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[1] Borgen, D. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **119**, 725.

TABLE I.
Ferrous and Ferric Iron in Liver Extract.

| No. and description of sample | | Total reducing power equivalent to mg $Fe^{++}$/cc | Total iron present, mg/cc* | Ferrous iron % of total iron |
|---|---|---|---|---|
| Z-34 | Ferrous Sulphate added | 20.5 | 6.35 | 97.3 |
| F-34S | ’’ ’’ ’’ | 24.4 | 8.12 | 98.0 |
| G-34S | ’’ ’’ ’’ | 23.5 | 8.32 | 96.3 |
| G-35S | ’’ ’’ ’’ | 24.4 | 7.63 | 99.0 |
| L-36S | ’’ ’’ ’’ | 17.2 | 8.04 | 98.0 |
| Q-36S | ’’ ’’ ’’ | — | 9.67 | 98.6 |
| I-35 | Ferric Ammonium Citrate added | 29.1 | 13.5 approx. | 90.0 |
| K-35 | ’’ ’’ ’’ ’’ | 31.4 | ’’ | 95.0 |
| L-36 | ’’ ’’ ’’ ’’ | 33.3 | ’’ | 77.0 |
| S-38 | ’’ ’’ ’’ ’’ | 27.2 | ’’ | 58.0 |
| B-39 | ’’ ’’ ’’ ’’ | 30.5 | ’’ | 66.0 |
| K-40 | ’’ ’’ ’’ ’’ | 22.5 | ’’ | 51.0 |
| T-40 | ’’ ’’ ’’ ’’ | 24.3 | ’’ | 70.0 |
| S-40 | ’’ ’’ ’’ ’’ | 22.8 | ’’ | 61.0 |
| C-40 | ’’ ’’ ’’ ’’ | 23.6 | ’’ | 66.0 |

*Each cc of extract was derived from about 7.5 g of liver, and, before the addition of iron, had a specific gravity of 1.25 at 60°F.

gested the possibility that liver extract might prevent the oxidation of ferrous iron added to it and thus furnish a stable solution of ferrous iron for oral ingestion, at the same time combining any advantages inherent in liver therapy.

*Procedure.* In order to estimate the total reducing power of the liver extract used in this study, a titrimetric method was used, employing as oxidation-reduction indicator the Ortho-Phenanthroline Ferrous Complex and as oxidizing agent, a ceric sulphate solution. This method estimates all ferrous ions and in addition any other material whose reducing potential is less than 1.14 volts. As will be evident in Table I the total reducing power of the solution consists of that due to the ferrous ions present and an additional amount due to reducing substances in the liver extract itself. The results are expressed in terms of equivalent amounts of ferrous iron.

The determination of ferrous and ferric iron was carried out according to McFarlane,[2] with *αα* Bipyridine and titanous chloride. The liver extract used throughout was a simple aqueous extract of pork liver. To 6 samples of this extract, processed on different dates from different purchases of liver, ferrous sulphate was added. Two to 3 weeks later analyses were made for ferrous and ferric iron. The results (Table I) show that the liver extract had effectively prevented oxidation.

The results of analyses for total reducing power suggested the possibility that ferric iron might be reduced to ferrous by the liver extract. A series of samples of commercial liver extract to which ferric ammonium citrate had been added* was analyzed for ferrous and ferric iron. The results (Table I) showed that 50 to 95% of the added iron had been reduced and was present as ferrous iron. The age of samples when analyzed varied from one month (C-40) to 4½ years (I-35).

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[2] McFarlane, William D., *J. Ind. Eng. Chem.*, 1936, **8**, 124.

Witts[3] has referred to the "potentiating action of liver" in the therapeutic use of iron in secondary anemias. Barker and Miller,[4] Cheney,[5] and other investigators[6, 7] have presented results which indicate that liver extract is a helpful therapeutic agent in the treatment of many cases of hypochromic anemia. The obvious suggestion arising from our experiments is that the reducing action of liver extract demonstrated here, by supplying or maintaining iron in the more efficiently utilizable ferrous state, may be, in part, the mechanism by which oral liver extract exerts whatever "potentiating" action it may have when used in combination with iron in the therapy of secondary anemias.

*Summary.* 1. An aqueous liver extract effectively prevented the oxidation of added ferrous iron. 2. Due to the reducing action of a commercial liver extract, from 50 to 95% of ferric iron added to it was later present in the ferrous state. 3. The efficacy of liver and iron combinations in the treatment of the iron deficiency anemias may be partly due to this action of the liver extract in supplying or maintaining iron in the more easily absorbable ferrous state.

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* Solution Liver Extract with Iron Valentine.

[3] Witts, L. J., *Lancet*, 1936, **1**, 1.

[4] Barker, W. H., and Miller, D. K., *Am. J. Med. Sc.*, 1938, **195**, 287.

[5] Cheney, G., *Folia Hemat.*, 1934, **52**, 31.

[6] Sturgis, C. C., and Farrar, G. E., Jr., *J. Exp. Med.*, 1935, **62**, 457.

[7] Keefer, C. S., and Yang, C. S., *Arch. Int. Med.*, 1931, **48**, 537.

### 11941

# Thermal Inhibition of Phage Production in Mixture of Phage and Growing Susceptible Bacteria.*

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*From the Department of Bacteriology, University of California.*

One of the most interesting aspects of the entire phage problem has been the question of how phage is formed. In earlier studies it appeared that phage was produced only in the presence of an actively growing bacterial substrate. This relationship found experimental verification in quantitative studies on the phage-bacterium reaction and led to the adoption of an equation in which phage production was expressed in terms of bacterial reproduction.[1] Further work, however, proved this relationship to be more apparent than real for it was found possible to impose conditions such that cell division was inhibited while phage continued to be developed at a very considerable rate.[2, 3, 4] The fact that phage can be produced at all in the absence of cellular reproduction indicated that the latter is not an essential conditioning factor for phage formation, even though the two phenomena ordinarily proceed concurrently and require careful adjustment of pH, temperature, electrolyte concentration,[5, 6] etc., to be dissociated.

Attention was then devoted to the possibility that phage production might involve the autocatalytic transformation of an inactive precursor into phage.[7] Formal proof of the existence of the precursor in cell-free solution has not yet been obtained, although traces can be detected irregularly.[8] However, there is satisfactory evidence that the substance from which phage is developed exists within susceptible cells.[9] To demonstrate it, staphylococci are subjected to active growth in an oxygenated medium, after which they are washed and

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*The authors wish to acknowledge the assistance accorded by grants-in-aid of research made by the John and Mary R. Markle Foundation and by Mr. Oscar Johnson.

[1] Krueger, A. P., and Northrop, J. H., *J. Gen. Physiol.*, 1930, **14**, 223.

[2] Krueger, A. P., and Fong, J., *J. Gen. Physiol.*, 1937, **21**, 2.

[3] Northrop, J. H., *J. Gen. Physiol.*, 1939-40, **23**, 59.

[4] Ellis, E. L., and Spizizen, J., *Science*, 1940, **92**, 91.

[5] Scribner, E. J., and Krueger, A. P., *J. Gen. Physiol.*, 1937, **21**, 1.

[6] Krueger, A. P., and Strietmann, W. L., *J. Gen. Physiol.*, 1938, **22**, 131.

[7] Krueger, A. P., *Science*, 1937, **86**, 379.

[8] Krueger, A. P., and Baldwin, D. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 393.

[9] Krueger, A. P., and Mundell, J. H., *Science*, 1938, **88**, 550.

stored in Locke's solution at 5°C. These "activated" cells when mixed with phage in appropriate proportions, bring about a tenfold increase in phage titre within 2 minutes.[10] The reaction does not implicate any obvious cellular activity beyond supplying preformed precursor, for the bacteria are in a resting state and hence are metabolically inactive. As a further precaution, the amounts of phage and activated bacteria can be adjusted to the critical lytic ratio, resulting in destruction of the cells and their complete removal from the reacting mixture.[11]

Experimental work has shown that the intracellular precursor is much more labile than the reproductive mechanism of the bacteria. For example, amounts of iodoacetic acid[12] and methylene blue[13] which will totally abolish the phage-augmenting effect of activated cells, are insufficient to interfere with their reproductive capacity. This is also true of heat inactivation;[14] the precursor is rapidly destroyed at temperatures ranging from 45°C to 50°C, although at these temperatures resting, precursor-containing cells fail to show any reduction in plate count. Further, the thermal inactivation reaction has a high critical thermal increment, $\mu$ being 90,000. This would suggest that the precursor, like phage itself, is a protein.

If the precursor theory is correct and if bacterial growth and phage formation are not related as cause and effect, it should be possible to select an environment for the phage-bacterium reaction in which cellular reproduction progresses normally while phage formation does not occur either because of destruction of precursor or inhibition of precursor synthesis. In unreported experiments by Krueger, Scribner and Mecracken, we attempted to accomplish this by exposing mixtures of growing bacteria and phage to the action of known precursor inactivators, *e.g.*, iodoacetic acid, KCN, and methylene blue + light. But in every case tested, the concentration of chemical required for inactivation of precursor also interfered with bacterial growth. More recently we have obtained positive results by the simpler procedure of carefully selecting the temperature, thereby avoiding certain technical difficulties inherent in the use of chemicals.

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[10] Krueger, A. P., and Scribner, E. J., *J. Gen. Physiol.*, 1938-39, **22**, 699.

[11] Krueger, A. P., and Scribner, E. J., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 51.

[12] Krueger, A. P., and Scribner, E. J., Proc. Soc. Exp. Biol. and Med., 1940, **43**, 416.

[13] Krueger, A. P., Scribner, E. J., and Mecracken, T., *J. Gen. Physiol.*, 1940, **23**, 705.

[14] Krueger, A. P., Mecracken, T., and Scribner, E. J., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 573.

A mixture of phage and staphylococci in nutrient broth of pH 7.4 containing 5 × $10^7$ bacteria/ml and 1 × $10^7$ phage units/ml was placed in the water bath shaker at 42.3°C. Samples were removed every 0.25 hours for determining:

a. [Total bacteria] (nephelometer).
b. [Viable bacteria] (plate count).
c. [Phage].

The turbidity measurements were conducted with the Klett-Summerson photoelectric nephelometer and the numbers of viable cells were determined by the standard plate count method. The phage content of serial dilutions of each sample was determined by the activity titration procedure.[15] As controls there were included:

a. Suspensions of staphylococci activated by the customary technic and subsequently maintained along with the experimental mixtures at 42.3°C to observe the effect of this temperature on pre-formed precursor.

b. Standard phage solution in broth kept at 42.3°C to detect possible thermal inactivation.

The results of 4 experiments are averaged in Fig. 1. Within 1.5 hours [bacteria] increased 450%, the viable count being approximately 65% of the total count. During this period there was no

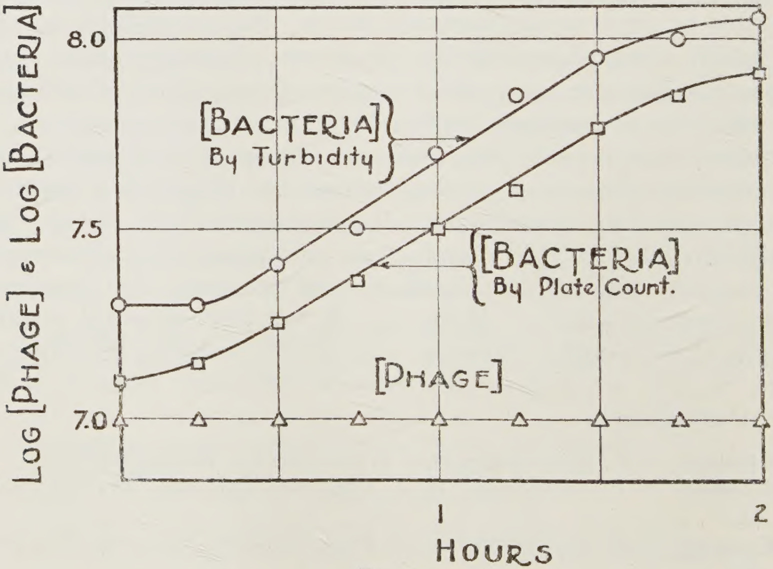

FIG. 1.
[Bacteria] and [Phage] in a broth mixture kept at 42.3°C.

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[15] Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 557.

detectable rise in [phage] and consequently the suspension never lysed. This maintenance of a constant titre cannot be ascribed to continuous thermal inactivation of phage just sufficient to balance the production of new phage, for in keeping with earlier work the control solutions held at 42.3°C showed no drop at the end of 2 hours. There remain two alternative explanations for the phenomenon: it may depend upon the thermal inactivation of precursor as it is produced in the growing organisms or it may involve inhibition of the synthetic mechanism. The first possibility appears remote because it has been shown in experiments on activated resting cells that the rate of precursor inactivation is relatively slow. The velocity constant at 42.3°C is 0.031 and it takes almost 1 hour to abolish the store of precursor in fully activated bacteria under the conditions of the present experiment.

Experimentally the fact is that when a mixture of phage and growing susceptible staphylococci is kept at 42.3°C the bacteria grow without inducing the formation of phage. The conclusion seems valid that this result depends upon inhibition of the precursor-producing mechanism of the cell rather than upon thermal destruction of phage or phage-precursor.

### 11942

## Nature of the Blood Iodine. II. Nature of the Plasma Iodine.

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*From the Laboratories of the Mount Sinai Hospital, New York City.*

In a previous publication[1] it was indicated that all the iodine normally occurring in the blood is in a form which does not pass through a cellophane membrane when the blood is dialyzed against water.

This study is an attempt to cast further light upon the state in which the iodine exists in the plasma.

It has been known since the early work of Baumann and Oswald that much of the iodine present in the thyroid gland exists in combination with protein, particularly globulin. The presence of a specific iodized globulin—thyroglobulin—has been established. Because of the presence of thyroglobulin as the chief iodine-containing protein in the thyroid gland it has often been assumed that this is the form in which the iodine circulates in the peripheral blood.

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[1] Silver, S., *J. Mt. Sinai Hosp.*, 1940, **7**, 97.

As early as 1923 Hektoen[2] attempted to demonstrate the presence of thyroglobulin in the circulating blood by means of a specific precipitin reaction. Recently these experiments have been repeated by Lerman[3] and by Stellar and Olken,[4] all of whom were unable to detect thyroglobulin either in the peripheral blood, the venous blood from the thyroid gland or in the lymph from the thyroid.

This study is a quantitative estimation of the plasma iodine and its distribution in the albumin and globulin fractions of the plasma.

*Method.* Sufficient pooled human plasma was obtained to allow for determinations in quadruplicate in each series. The total iodine in the plasma was determined using a modification of the Leipert procedure as modified by Matthews.[5] The plasma was then divided into albumin and globulin fractions by salting out with half-saturated ammonium sulfate. The globulin fraction was washed with half-saturated ammonium sulfate and these washings were added to the albumin fraction. The iodine content of each fraction was determined separately. All the usual precautions to insure iodine-free reagents were taken. The blank was less than 0.04 gamma iodine per determination.

The total plasma iodine was determined using 25 cc of plasma. For the albumin and globulin determinations 50 cc of plasma was treated with 50 cc of saturated ammonium sulfate.

*Discussion.* If thyroglobulin is the chief form in which the iodine circulates then most of the plasma iodine should be in the globulin fraction. This, however, is not the case. There is evidence, rather, that the albumin and globulin fractions each contain iodine and that the iodine content of each fraction varies with the protein content of the fraction. There is no evidence to assume that thyroglobulin is the main form in which the blood iodine circulates.

It seems, therefore, that although thyroglobulin may be the form in which the hormone is *stored* in the thyroid gland it is not the only form in which it circulates. It would, in fact, be difficult to visualize how so large a molecule could pass through the cells of the gland and the blood vessels to reach the circulating blood. It is more likely that the stored thyroglobulin is fragmented, and iodized amino acids, short chain iodized polypeptids or other iodized frag-

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[2] Hektoen, L., Carlson, A. J., and Schulof, K., *J. A. M. A.*, 1923, **81**, 86.

[3] Lerman, J., *J. Clin. Invest.*, 1940, **19**, 555.

[4] Stellar, L. I., and Olken, H. G., *Endocrinology*, 1940, **27**, 614.

[5] Matthews, N. L., *et al.*, *J. Indust. Chem.*, 1938, **10**, 612.

TABLE I.
Results: Iodine Content of Pooled Plasma and Its Fractions
All iodine values in micrograms.

| | Plasma 1 | Plasma 2 | Plasma 3 |
|---|---|---|---|
| Iodine in 50 cc plasma | 3.5 | 2.6 | 2.6 |
| | 3.7 | 2.6 | 3.0 |
| | 3.5 | 2.3 | 3.0 |
| | 3.6 | 2.4 | 3.1 |
| Avg | 3.6 | 2.5 | 2.9 |
| Iodine in albumin fraction derived from 50 cc plasma | 2.7 | 1.9 | 2.1 |
| | 2.4 | 2.1 | 2.0 |
| | 2.5 | 1.7 | 2.2 |
| | 2.5 | 1.9 | 2.3 |
| Avg | 2.5 | 1.9 | 2.1 |
| Iodine in globulin fraction derived from 50 cc plasma | 1.2 | 1.0 | 1.2 |
| | 1.3 | 1.2 | 1.0 |
| | 1.3 | 1.1 | 1.0 |
| | 1.3 | 1.1 | 0.9 |
| Avg | 1.3 | 1.1 | 1.0 |
| % total protein in plasma | 6.8 | 6.3 | 6.1 |
| Albumin-globulin ratio in plasma | 2.1-1 | 2.0-1 | 2.2-1 |
| Parts iodine per million in albumin fraction | 1.1 | 0.9 | 1.0 |
| Parts iodine per million in globulin fraction | 1.2 | 1.0 | 1.0 |

ments of thyroglobulin are secreted into the blood stream and that these are then incorporated in the circulating serum proteins without regard to the specific nature of these proteins, but rather in proportion to the concentrations of these proteins in the plasma. Unpublished studies have shown that both albumin and globulin *in vitro* have the ability to fix thyroxine and diiodotyrosine and render them non-dialyzable.

*Summary.* The plasma iodine is divided between the albumin and the globulin fractions of the plasma in proportion to the relative amounts of albumin and globulin present. There is no chemical evidence for the presence of a preponderant amount of thyroglobulin in the circulating blood.

### 11943 P

# Urinary Citrates in Calcium Urolithiasis.

BEN KISSIN AND M. OWEN LOCKS. (Introduced by B. Kramer.)
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The existence of a soluble, non-ionized calcium citrate complex has been established by the work of many investigators.[1-7] The dissociation of calcium citrate is given by Hastings, McLean, *et al.*:[8]

$$Ca_3Cit_2 \rightarrow Ca^{++} + 2CaCit^- \qquad (1)$$

$$CaCit^- \rightleftharpoons Ca^{++} + Cit^{\equiv} \qquad (2)$$

Thus, in equation 2, the more citrate present, the more $CaCit^-$ complex is formed and the less calcium is present in the form of calcium ion ($Ca^{++}$).

The equilibrium of an insoluble calcium salt, as for example $CaCO_3$, in a water system, may be expressed as:

$$\mathbf{CaCO_3\ (precipitate) \rightleftharpoons Ca^{++} + CO_3^{=}\ (in\ solution)} \qquad (3)$$

The addition of citrate ion ($Cit^{\equiv}$) to the system removes the $Ca^{++}$ by uniting with it, as in equation 2, to form $CaCit^-$, thus displacing equation 3 to the right. This is equivalent to an increased solubility of the relatively insoluble salt. Greenwald[9] determined the exact increases in apparent solubility product of $Ca_3(PO_4)_2$ and $CaCO_3$ with a given citrate concentration, and found large increases in solubility with small concentrations of citrate. Albright, Sulkowitch and Chute[10] demonstrated the solution of $Ca_3(PO_4)_2$ stones *in vitro* and *in vivo* with alkaline citrate solutions.

The daily excretion of citrates in the urine of normal individuals as determined by Ostberg[11] varied from 140 mg to 1,340 mg expressed in terms of citric acid. However, these were the extreme variations and the great majority of the determinations were close to the average of 530 mg. This is a larger concentration than that

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[1] Greenwald, *J. Biol. Chem.*, 1926, **67**, 1.

[2] Hastings, Murray and Sendroy, *J. Biol. Chem.*, 1926-27, **71**, 723.

[3] Clark, Percival and Stewart, *J. Physiol.*, 1928, **66**, 346.

[4] Shelling and Maslow, *J. Biol. Chem.*, 1928, **78**, 661.

[5] Shear and Kramer, *J. Biol. Chem.*, 1928, **79**, 161.

[6] Shear, Kramer and Reznikoff, *J. Biol. Chem.*, 1929, **83**, 721.

[7] Whittier, *J. Biol. Chem.*, 1938, **123**, 283.

[8] Hastings, McLean, Eichelberger, Hall and DaCosta, *J. Biol. Chem.*, 1934, **107**, 351.

[9] Greenwald, *J. Biol. Chem.*, 1938, **124**, 437.

[10] Albright, Sulkowitch and Chute, *J. Am. Med. Assn.*, 1939, **113**, 2049.

[11] Ostberg, *Skand. Arch. Physiol.*, 1931, **62**, 81.

used by Greenwald[9] and should have some effect on the solubility of calcium in urine. The purpose of this experiment was to determine the daily urinary excretion of citrates in patients with calcium urolithiasis to see whether this condition might be associated with a decrease in that component.

*Method.* Sixteen cases of calcium urolithiasis, proven by X-ray or postoperative analysis, were chosen from 4 hospitals. All patients were on normal house diets with little or no medication. Sixteen other patients were carefully picked as controls from the same hospitals, an effort being made to match the lithiasis patients as to age, sex, diet and bed regimen. In most instances each patient had a comparable control. Twenty-four-hour specimens of urine were collected under toluene, and determinations of citrate made according to the method of Pucher, Sherman and Vickery.[12]

To check the reliability of the method used, determinations were made on solutions containing known amounts of citric acid. In addition, quantities of citric acid corresponding to the amounts found in urine were added to samples of urine. Our analyses showed quantitative recovery within the limits of error reported by the authors (Pucher, Sherman and Vickery). In Table I we give the concentration of citrate and the 24-hour excretion in the urines of 16 patients suffering with calcium stones and in an equal number of patients who, clinically and by X-ray, were found to be free of such stones.

TABLE I.
The 24-hour Citric Acid Excretion of Ca Stone Patients and Controls.

| Patient | Conc. HCit, mg/cc | Total HCit, mg | Control | Conc. HCit, mg/cc | Total HCit, mg |
|---|---|---|---|---|---|
| 1 | .02 | 30 | 1 | .12 | 180 |
| 2 | .03 | 45 | 2 | .08 | 192 |
| 3 | .10 | 57.5 | 3 | .19 | 270 |
| 4 | .04 | 71.2 | 4 | .20 | 300 |
| 5 | .13 | 84.5 | 5 | .17 | 438 |
| 6 | .08 | 120 | 6 | .88 | 485 |
| 7 | .08 | 144 | 7 | 1.65 | 495 |
| 8 | .08 | 152 | 8 | .30 | 525 |
| 9 | .17 | 153 | 9 | 1.10 | 550 |
| 10 | .06 | 162 | 10 | .98 | 637 |
| 11 | .12 | 192 | 11 | .57 | 855 |
| 12 | .12 | 204 | 12 | .48 | 864 |
| 13 | .09 | 216 | 13 | .49 | 906.5 |
| 14 | .18 | 270 | 14 | .64 | 1024 |
| 15 | .55 | 302 | 15 | 1.01 | 1060.5 |
| 16 | .19 | 342 | 16 | .84 | 1260 |
| Avg | .13 | 165.3 | | .61 | 627.6 |

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[12] Pucher, Sherman and Vickery, *J. Biol. Chem.*, 1936, **113**, 235.

*Discussion.* According to these determinations, the concentration of citric acid in the urine of calcium urolithiasis patients is, on the whole, exceptionally low. Fifteen of the 16 cases excreted less than .20 mg per cc of urine. In the control group, only 5 cases excreted less than .20 mg per cc. The average concentration for the patients was .13 mg per cc and that for the controls, .61 mg per cc. As regards the total daily citric acid output, all the calcium stone patients excreted less than 350 mg, 11 patients with less than 200 mg. Only 4 of the controls excreted less than 350 mg daily, the others ranging much higher. The average daily excretion for the patients was 165.3 mg, while that of the control group was 627.6 mg.

The spread of the values among the controls compares well with that found by Ostberg for normal individuals. It compares well, also, with the results of Sherman, Mendel and Smith,[13] who, with the same method used in this paper, found the normal spread to be from 356 mg to 1,180 mg daily. The fact that only one determination was made for each patient is probably not of very great significance since Ostberg found a marked tendency for these values to remain about the same from day to day.

The value of this experiment lies in the fact that it presents a possible etiological factor in the formation of calcium urinary stones. Furthermore, increasing the citrate concentration of urine, by the administration of citrates or of dicarboxylic acids which, according to Orten and Smith,[14] are converted to urinary citrates, may offer a new prophylactic or even therapeutic measure.

The authors express their thanks to Dr. Benjamin Kramer, who suggested this problem and under whose supervision the work was done.

*Conclusions.* The daily urinary citrate content and concentration of patients with calcium urolithiasis is much lower than those of normal individuals. This finding may be of etiological importance.

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[13] Sherman, Mendel and Smith, *J. Biol. Chem.*, 1936, **113**, 247.

[14] Orten and Smith, *J. Biol. Chem.*, 1937, **117**, 555.

### 11944 P

# Effects of CO and Methylene Blue upon $O_2$ Consumption of Shark (Mustelus) Blood.*

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The purpose of this experiment was to see if methylene blue had any effect upon the $O_2$ consumption of shark *(Mustelus)* red blood cells in the presence of carbon monoxide or air. Since these red blood cells are nucleated and have a considerable rate of respiration, it was thought of interest to test Barron's conclusions that the action of methylene blue in increasing oxygen consumption is less marked, or even *nil,*[1] in nucleated erythrocytes or leucocytes as contrasted with such non-nucleated erythrocytes as mammalian. In addition it was also thought of interest to find out whether methylene blue antagonizes the effects of CO on the respiration of these nucleated erythrocytes.

The blood was collected fresh for each experiment into tubes by cutting the dorsal posterior fin of the shark. In some experiments the blood was centrifuged and the erythrocytes were subsequently washed several times in 1.1% NaCl and in others whole blood was used. No difference in the results was noted. Aliquot portions of 2 cc of blood were placed in the bottom of cups of Warburg respiration apparatus, temp. 20°C. Either 100% CO‡ or air was used in the system. $CO_2$ was absorbed by KOH in the insets in the usual way. The concentration of methylene blue added was .004% made in 1.1% NaCl. Tests were made to see if complete darkness affected the results with CO. No such effects were noted. The material was found to be remarkably uniform in behavior, and remarkably resistant to hemolysis in a wide range of NaCl concentrations.

The results are shown in Table I.

This table shows (1) that *Mustelus* blood has a considerable rate

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* Aid from a grant from John Wyeth & Bros. is herewith gratefully acknowledged.

† With the technical assistance of R. R. Ronkin.

[1] Barron, *J. Biol Chem.*, 1929, **81**, 445, states that the action of methylene blue was nil when chicken blood was used.

‡ CO was prepared by dropping formic acid into hot concentrated $H_2SO_4$ and leading the gas through soda lime.

TABLE I.
Mm$^3$ $O_2$ Consumed in 2 to 4 Hours by *Mustelus* Blood.

| | In air | In CO | In air | In CO |
|---|---|---|---|---|
| Added | Nothing | Nothing | Methylene blue | Methylene blue |
| No. of exp. | 15 | 12 | 9 | 8 |
| $O_2$ consumed (in mm$^3$) | 23.3 | 14.4 | 35.2 | 20.1 |
| ” ” % | 100 ± 2.7% | 61.8 ± 7.2% | 151 ± 5.0% | 86.1 ± 4.3% |

of respiration: (2) that in air methylene blue causes an increased $O_2$ consumption of 151% over the controls in 2 to 4 hours under the conditions of the experiment—this does not confirm Barron's conclusions that methylene blue has little or not effect upon the respiration of nucleated red blood cells; (3) that CO produces a decrease to 61% (it was not determined in these experiments whether this change in the manometric readings was due to residual oxygen remaining after the atmosphere was saturated with CO, or actual CO consumption); (4) that methylene blue counteracts this decrease, increasing the rate to 86%. This increased rate in CO is about the same in percent as that produced by methylene blue in air.

## 11945

## Oral and Intramuscular Ineffectiveness of "Hypotensive" Extracts.

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Administered intravenously in animals, the commonly used "hypotensive" extracts obtained from the pancreas, muscles and urine possess definite, though fleeting and complex, hemodynamic actions, with marked individual and species differences.[1, 2] However, the therapeutic claims for these extracts in circulatory disorders (Raynaud's disease, Buerger's disease, hypertension, angina pectoris, intermittent claudication, etc.) are based on oral and intramuscular administrations. Therefore, it was thought worth while to in-

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[1] Lehman, A. J., and Van Winkle, W., Jr., *Arch. internat. de pharm. et de therap.*, 1938, **59**, 75.

[2] Van Winkle, W., Jr., and Lehman, A. J., *Arch. internat. de pharm. et de therap.*, 1938, **59**, 133.

vestigate the oral and intramuscular absorbability of representative products.

*Absorption.* Six pigeons, 7 rabbits, 7 cats and 2 dogs were used. Anesthesia was obtained with pentobarbital, 35 mg per kg intraperitoneally. Blood pressure was recorded in the usual manner from the carotid artery. Intramuscular injections were made into the pectoral muscles of pigeons and gluteal muscles of mammals. The injected region was massaged to promote absorption. The criterion of absorption in mammals was a fall of blood pressure (depressor effect), and in pigeons, a rise (pressor effect). The two extracts used were padutin (kallikrein; pancreas or urine)* and tissue extract No. 568 (pancreas),† in doses far exceeding those recommended as therapeutic.

Briefly, all animals reacted typically to intravenous injections of the extracts, in doses of 0.1 cc per kg, both before and after oral and intramuscular administrations. However, in no case was absorption of these extracts demonstrable after repeated oral and intramuscular administrations, as indicated by an absence of blood pressure changes and symptoms. More than one hour was allowed for any effects, and doses as high as 5 cc per kg were tried intramuscularly and orally, *i. e.,* 50 times the effective intravenous dose.

Direct absorption from the intestine (in the absence of destructive effects of digestive enzymes) was tested by injecting the extracts into ligated empty loops of small intestine of dogs and cats, using about a 20 cm length of jejunum. Two hours were allowed for absorption, and 2 cc per kg of the extracts were injected. Again, there were no demonstrable changes in blood pressure. At the end of 2 hours, the contents of the loop were removed and 1/10 of the volume was injected intravenously into the same animal. Invariably, the blood pressure fell about 50% in all animals receiving both extracts. Intravenous injection of the same volume of contents from another, and similar, intestinal loop containing 0.9% sodium chloride solution, as control, caused a fleeting and negligible fall of blood pressure of only about 6%. Intravenous injections of 0.2 cc per kg of the tissue extracts themselves produced falls of blood pressure equal in degree and duration to those after injection of contents from loops which had received the extracts. Accordingly, the tissue extracts remained practically unchanged, qualitatively and quantitatively, in the intestinal loops and there was no demonstrable systemic absorption.

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* Marketed by Winthrop Chemical Co.

† Marketed by Sharpe and Dohme.

*Toxic and Fatal Doses.* Although gastrointestinal and intramuscular absorptions of the "hypotensive" extracts in high therapeutic doses were not demonstrable in anesthetized mammals and pigeons, a test was made for possible toxic effects in healthy and previously untreated pigeons. However, oral and intramuscular doses as high as 20 cc per kg produced no demonstrable effects in 11 pigeons and all the birds remained in good condition. The highest dose tried, or 20 cc per kg, was the equivalent of giving at once 1.4 liters of the extracts by the same routes to a 70 kg man, or about 300 to 1400 times a single recommended therapeutic dose.

Injected intravenously, the same pigeons used for tests of oral and intramuscular toxicity and other previously untreated pigeons (total 41) reacted to the extracts in percent mortality and dose per kg as follows: tissue extract No. 568: 100%, 12.5 cc; 60%, 7.5 cc; 33%, 5 cc; none, 2.5 cc; padutin: 100%, 10 cc; 60%, 9 cc; 40%, 8.5 cc; none, 7.5 cc. Death, in fatal cases, occurred within 5 minutes, preceded by tremors, emesis, increased respiration, opisthotonus, convulsions and coma; respiration stopped before the heart. Clearly, intravenous injections of these "hypotensive" extracts are capable of producing violent symptoms, and even death, but the doses would have to be beyond the therapeutic. In sensitive persons, however, the dose might be smaller. Such effects could conceivably occur from accidental intravenous injections in the intramuscular or hypodermic use of these extracts.

*Summary.* Two hypotensive extracts, namely Padutin and tissue extract No. 568, advocated in the treatment of important circulatory disorders, produced no demonstrable changes in blood pressure and no symptoms after oral and intramuscular administrations in very high (beyond therapeutic) doses in animals. Placed directly into ligated intestinal loops, both extracts remained unchanged and unabsorbed for 2 hours. All the animals reacted typically to control intravenous injections of both extracts. A range of fatal doses was determined intravenously only in pigeons, because very much larger doses given either by the oral or intramuscular route were without demonstrable effects. An experimental basis for the clinical use of the "hypotensive" tissue extracts, either orally or intramuscularly, is completely lacking. However, accidental intravenous injections, resulting from careless intramuscular or hypodermic uses, could conceivably cause alarming symptoms and even death. These extracts are undesirable, if not dangerous, for intravenous use.

### 11946 P

## Quinine Hypersensitivity in Guinea Pigs.

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In an attempt to widen the range of compounds amenable to experimentation on hypersensitiveness in animals, using substances which may differ in the mechanism of sensitization, we undertook to sensitize guinea pigs with quinine which is known to cause allergy in human beings. Some positive sensitization results of moderate degree were obtained after a number of applications to the skin of a 5% solution in a mixture of one part of butyl carbitol (diethylene glycol monobutyl ether) and 3 parts olive oil. In several experiments in which this treatment was made instead on inflamed areas the number and degree of positive effects were increased; inflammation was produced with cantharidin, similar to previous work,[1] along with other irritants (xylene, croton oil, etc.). Likewise, marked sensitivity was achieved when quinine was applied to areas inflamed by 2:4 dinitrochlorobenzene in animals sensitive to the latter substance.

In well sensitized animals the reactions following the application of one drop of the above quinine solution are well developed by the following day and consist of intense reddening and slight elevation of the treated area. In less sensitive animals pronounced reactions appeared after 2 or 3 consecutive daily applications to the same site.

In a preliminary experiment the method of cantharidin burns gave positive sensitization effects also with a nickel salt (sulfate).

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### 11947

## Immunizing Value of Rickettsial Vaccines.

TIMOTHY J. KUROTCHKIN AND RALPH W. G. WYCKOFF.
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Recently developed methods for the cultivation of Rocky Mountain spotted fever and typhus rickettsiae in agar slant tissue cultures[1] and in the yolk sac[2] of the developing chick embryo have opened new possibilities for the preparation of vaccines against these infections. We have been making comparative studies (a) of the immunizing potencies of vaccines obtained from rickettsiae grown in these ways and (b) of the relative merits of these procedures for the large scale production of vaccines.

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[1] Landsteiner, K., and DiSomma, A. A., *J. Exp. Med.*, 1940, **72**, 361.

[1] Zinsser, H., Wei, H., and Fitzpatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 604; *J. Exp. Med.*, 1939, **69**, 179.

These comparative tests have been made using an "eastern" strain of spotted fever rickettsiae of relatively low virulence. It had been maintained in agar tissue cultures for at least one year before being used in the present experiments. It was uniformly infectious for guinea pigs but unlike more virulent strains of spotted fever the mortality rate among diseased pigs was not high. In spite of this difference in virulence vaccines made from it protected guinea pigs against many lethal doses of "western" spotted fever rickettsiae, and vice versa.

For the production of vaccines[3] this rickettsial strain was carried both on agar slant cultures and in developing eggs. When grown in culture large test tubes containing Tyrode-serum agar slants were inoculated with infected tissue according to the technic described by Zinsser, Wei and Fitzpatrick.[1] The tubes were incubated at 35°C for 5, 6, or 7 days. At the end of this period smears were made from bits of tissue detached from the agar slants, strained and examined for rickettsiae. Cultures showing good growth of rickettsiae were harvested by scraping off the tissue with a small spatula, care being taken not to disturb the agar surface itself. This was then used immediately for the production of vaccine. It was early observed that many rickettsiae could be demonstrated in smears prepared by scratching the surface of the agar slants after this removal of tissue; and it was thought that several crops of infected tissue might be obtained from the same slant by merely reseeding the harvested tube with fresh embryonic pulp. This was the case. Therefore, after harvesting its infected tissue a slant was allowed to absorb carbon dioxide to neutralize the acidity it had developed as a result of the previous tissue metabolism; it was then seeded with fresh pulp and incubated to produce a new crop of infected tissue. This was continued as long as the rickettsial yield remained high and no bacterial contamination occurred, as shown by microscopic examination made at the end of the incubation period. Usually a slant was reinoculated 3 or 4 times in this fashion before being discarded. This process of reinoculation reduced very materially the cost of vaccine production, both in time and in cost of nutrient medium. To make vaccine the infected tissue taken from the agar slants was weighed and thoroughly ground. A phosphate-saline buffer solution of pH 7.0 containing 0.25% formalin and 0.25% phenol was added to this mass in an amount sufficient to make a 2% suspension of tissue. The buffer solution was 0.05M with respect to phosphate ions and 0.15M with respect to NaCl. Crude vaccine thus prepared was inactivated by being kept for 48 hours at 70°F and for at least 5 days in the chill room before use. At the conclusion of the inactivating period it was lightly centrifuged to remove any large clumps of tissue that remained. About 10 liters of Zinsser-type vaccine were made in this fashion in 5 separate batches. Samples of each of these were separately tested for potency in the comparative tests to be described below.

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[2] Cox, H. R., *U. S. Public Health Rep.*, 1938, **53**, 2241; 1939, **54**, 1070.

[3] Zinsser, H., Plotz, H., and Enders, J. F., *Science*, 1940, **91**, 51.

The procedure used in preparing vaccine according to the yolk sac method of Cox was as follows: Fertile eggs which had been incubated for 7 to 8 days were inoculated by injecting 0.5 cc of highly infectious rickettsial suspension directly into the yolk sac of each egg. Nearly all embryos were moribund in from 3 to 4 days. Microscopic study of smears of yolk sac tissue from such embryos showed a considerable number of rickettsiae with smaller numbers in smears of the chorioallantoic membrane. Vaccine has been made from the combined yolk sac and chorioallantoic membranes taken from moribund embryos. In harvesting them these membranes, freed from the embryos and other egg contents, were placed on sterile wire mesh and excess of yolk was drained off under mild pressure. The infected membrane tissue was then weighed and thoroughly ground. Saline solution containing 0.3% formalin and 1% phenol was added to this ground mass in the proportion of 100 cc to every 10 g of tissue. This concentrated suspension was inactivated by being left for 48 hours at 70°F and 5 days in the chill room. It was then centrifuged for 45 minutes at 2000 r.p.m. The supernatant fluid was separated, filtered through 12 to 15 layers of sterile gauze, measured and diluted with enough saline solution to give the equivalent of a 2% suspension of the original tissue. This was the finished vaccine. Four different lots of vaccine made in this fashion were employed in the comparative potency tests.

The methods used in preparing these Zinsser and Cox type vaccines are similar but not exactly the same. Both were made 2% with respect to the original infected tissue. The centrifugal clarification was much less complete with the Zinsser vaccine; this should have favored its potency by retaining in it any rickettsiae still associated with tissue fragments. The Cox vaccine was inactivated with strong formalin and phenol; if these antiseptics had a deleterious effect on the antigenicity of the rickettsiae or their products they should have weakened, relatively, the immunizing capacity of the yolk sac vaccine.

The immunizing potencies of all lots of both types of vaccine have been tested in guinea pigs. To do this portions of each lot of vaccine were diluted with from 2 to 16 parts of saline solution. Series of guinea pigs were injected subcutaneously with one cc doses of these diluted vaccines. The vaccination of each animal was completed by giving a second equal injection after one week. Seven days after this second vaccination the immunity of the guinea pigs was determined by injecting them intraperitoneally with one cc of a 1:1000 dilution of infected yolk sac tissue rich in rickettsiae. In every experiment unvaccinated guinea pigs serving as controls were injected with this same virus dose; without exception these pigs developed characteristic fever. On 12 occasions we have made dilution titrations of the infectivity of yolk sac tissue diseased with the strain of spotted fever rickettsiae used in these experiments. The end point was always at the $1:10^6$ dilution so we may conclude that the challenge dose administered in the present experiments was of the order of 1000 guinea pig-infectious doses; no unvaccinated control guinea pig failed to become sick and develop typical fever after the injection of this dose of rickettsiae.

The daily temperatures of typical unvaccinated and vaccinated guinea pigs after challenge inoculation are recorded in Table I. The result of vaccine-protection studies with the 5 lots of Zinsser- and

TABLE I.
Temperatures (°F) of Guinea Pigs after Injection of Challenge Rickettsial Dose.

| Days after injection | Guinea Pig No.<br>Vaccine Dose | A<br>0.0cc | B<br>0.0cc | C<br>0.0cc | D<br>0.50cc | E<br>0.25cc | F<br>0.125cc | G<br>0.125cc |
|---|---|---|---|---|---|---|---|---|
| 1 | | 101.4° | 103.0° | 101.0° | 101.9° | 102.4° | 102.4° | 102.2° |
| 2 | | 120.4 | 103.3 | 103.2 | 100.8 | 102.2 | 102.0 | 101.0 |
| 3 | | 102.0 | 103.6 | 103.8 | 100.6 | 101.6 | 102.8 | 100.4 |
| 4 | | 103.8 | *105.4* | 102.9 | 101.2 | 101.2 | 103.0 | 101.4 |
| 5 | | *105.0* | *105.2* | *104.9* | 102.0 | 102.0 | 103.0 | 101.0 |
| 6 | | *105.4* | 105.5 | *105.0* | 102.6 | 101.6 | 102.6 | 100.8 |
| 7 | | 104.4 | 104.6 | 104.9 | 101.8 | 101.4 | *103.8* | 101.4 |
| 8 | | 105.1 | 104.3 | 104.4 | 100.4 | 101.8 | *104.0* | 101.4 |
| 9 | | 105.3 | 103.0 | 104.3 | 101.6 | 102.0 | 104.0 | 101.0 |
| 10 | | D | 104.1 | 103.8 | 102.2 | 102.4 | 104.0 | 101.2 |
| 11 | | — | 103.5 | 102.9 | 102.4 | 102.6 | 103.0 | 101.6 |
| 12 | | — | 103.7 | 102.0 | 102.6 | 102.4 | 103.2 | 101.8 |
| 13 | | — | D | — | 101.8 | 102.0 | S | 101.0 |

Note: In this table D indicates death and S survival. The point of onset of disease has been indicated by italicized daily temperatures. According to our evaluation animals D, E and G were completely, animal F was incompletely protected.

the 4 lots of Cox-type vaccine are collected in Table II. Taking the continued health of the animal and the absence of fever as index of protection it is obvious that all 9 batches of vaccine were able to convey a high degree of protection to guinea pigs. Zinsser vaccine protected all animals when a total of at least 0.25 cc of the original vaccines was administered. When the dose was reduced to 0.125 cc from 50 to 66% of the animals were fully protected. Three lots of Cox vaccine gave complete protection down through a total dose of 0.125 cc, corresponding to the lowest dilution tested. The fourth and weaker lot gave complete protection at 0.25 cc but not at 0.125 cc. From these experiments it would appear that the yolk sac vaccine is at least as effective an immunizing agent for guinea pigs as the tissue culture vaccine. We have made large volumes of vaccine by both methods and are convinced that under the conditions necessary

TABLE II.
Comparative Immunizing Values of Rickettsial Vaccines.

| Lot No. | No. of vaccinated animals | Total amt of vaccine, cc | No. of animals showing no fever | % Protection |
|---|---|---|---|---|
| 1Z | 4 | 1.00 | 4 | 100 |
| | 4 | 0.50 | 4 | 100 |
| | 3 | 0.25 | 3 | 100 |
| 2Z | 4 | 1.00 | 4 | 100 |
| | 4 | 0.50 | 4 | 100 |
| | 4 | 0.25 | 4 | 100 |
| | 3 | 0.125 | 2 | 66 |
| 3Z | 4 | 1.00 | 4 | 100 |
| | 4 | 0.50 | 4 | 100 |
| | 4 | 0.25 | 4 | 100 |
| 4Z | 4 | 0.25 | 4 | 100 |
| | 4 | 0.125 | 2 | 50 |
| 5Z | 4 | 0.25 | 4 | 100 |
| | 4 | 0.125 | 4 | 50 |
| 1C | 4 | 1.00 | 4 | 100 |
| | 4 | 0.25 | 4 | 100 |
| | 3 | 0.125 | 2 | 66 |
| 2C | 3 | 1.00 | 3 | 100 |
| | 4 | 0.25 | 4 | 100 |
| | 3 | 0.125 | 3 | 100 |
| 3C | 4 | 1.00 | 4 | 100 |
| | 4 | 0.25 | 4 | 100 |
| | 4 | 0.125 | 4 | 100 |
| 4C | 2 | 1.00 | 2 | 100 |
| | 4 | 0.25 | 4 | 100 |
| | 4 | 0.125 | 4 | 100 |

Note: In this table Z and C refer to vaccines made according to the agar slant and yolk sac methods of Zinsser and Cox respectively.

to produce vaccine in large amounts the yolk sac technic is also easier to carry out and much less costly in time and materials.

Though the foregoing experiments were made with an eastern strain of spotted fever rickettsiae, we have also experimented with more virulent strains of the disease and with the Breinl strain of epidemic typhus rickettsiae. From our experience we are convinced that the conclusions just expressed are equally applicable to problems of making vaccine against the diseases caused by these other rickettsiae.

### 11948

## Physiological and Toxicological Effects of Some Fish Muscle Extracts.

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While occasional descriptions of poisoning by fishes are encountered in ancient writings, surprisingly few scientific data of experimental nature on this subject are to be found in modern pharmacological literature. Of medicinal agents derived from fishes and mentioned in modern materia medica probably the best known is cod liver oil, which physicians used empirically long before discovery of vitamin D. Another "fish" product employed in therapeutics is spermacetti, well known ointment base. With the advent of the vitamin era an intensive search was naturally made for these substances in other fishes than the cod, *e. g.*, in the halibut, haddock and menhaden. Another interesting study of what may be called a drug rather than a toxic agent was that made by Macht and Barba-Gosé on the oil expressed from the flesh and bones of the *Ruvettus pretiosus*, popularly known as the "castor-oil" fish.[1] This investigation was of special interest in respect to the chemical nature of the laxative principles contained in the oil.[2] Other poisons (not of putrefactive origin) are found in various fishes but few researches of an experimental nature have been published concerning them. Here belong the contributions made by Mosso[3] on the toxicity of the blood serum of certain *Muraena*, the studies by MacCrudden[4] on the choleriform effects produced by the ovaries of the barbel and the curious findings made by Japanese investigators with regard to the neurotoxic effect of extracts from the gonads of certain species of *Tetraodontidae* and *Diodontidae*.[5, 6] With the exception of that made on extracts from the *Ruvettus pretiosus*, no specific pharmacological research on the effects of fish *muscle* has been recorded. In connection with an extensive study, pharmacological and toxicological, on different animal poisons, and on snake venoms in particular, carried on by the senior author and his associates,[7] it was deemed interesting to inquire into the pharmacological properties of muscle extracts obtained from various fishes. Accordingly the writers began to study the biological effects on both animal and plant test objects of muscle extracts derived from all kinds of fishes.

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[1] Macht and Barba-Gosé, *J. Am. Pharm. Assn.*, 1931, **20**, 558.

[2] Macht and Barba-Gosé, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 772.

[3] Mosso, *Arch. f. exp. Path.*, 1888, **25**, 111.

Experiments have already been made in this laboratory with *freshly expressed* muscle juices of 65 different varieties, and new species of fish are continually being sought and subjected to careful study. In every experiment, the effects of decomposition were absolutely excluded. All the fish were placed in cold storage within a few hours of their capture and frozen solid and were kept in that condition until just before the experiment was begun. A piece of muscle for a preparation of the extract was then sawed out and the rest of the fish was replaced in cold storage. By "extracts" is meant the juice expressed from fresh muscle tissue without the use of any chemical solvent, the suspension in every experiment being prepared as follows:

*Method of Preparation.* Five to 10 g of fresh fish muscle are carefully minced and ground with an equal weight of clean sand and with 0.8% of physiological sodium chloride solution, 2 cc being employed for each gram of muscle. The emulsion or suspension produced by such painstaking grinding of the tissue is then strained or pressed through fine muslin and the filtrate is employed for pharmacological tests.

*Zoo*pharmacological experiments were made by injecting intraperitoneally 1 to 3 cc into white mice weighing from 20 to 25 g each. *Phyto*pharmacological experiments were performed by the methods introduced by Macht and his collaborators and described in various papers published during the past 18 years.[8] This method, admirably suited to detection of presence of small quantities of animal poisons,[9] consists of studying the growth of *Lupinus albus* seedlings in plant-physiological solutions under standardized conditions described elsewhere. The increment or growth of the single straight roots of *Lupinus albus* seedlings is measured after the plants have been grown for 24 hours in the dark at 15°C or 60°F, and the growth of control seedlings in plant-physiological (Shive) solution is compared with the growth of similar seedlings from the same crop in 1% solutions of the muscle suspensions. The index of growth or phytotoxic index is expressed by the ratio of X/N or average growth in the unknown solution over the growth of the controls. The results obtained by means of the phytopharmacological test with 54 kinds of fresh muscle extracts are shown in Table I. The whole series of fish available was subjected to study under the standardized conditions described above. Most of the fish are of the edible type while others are not generally employed as food and still others are not eaten at all. It will be noted that some exerted a phytotoxic effect while others did not. Muscle suspensions made from all the non-poisonous and edible fishes gave indices of growth of not less than 70%. Most of the indices obtained were much higher, and some of the muscle extracts were actually stimulating for growth of the seedlings.

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[4] MacCrudden, *Arch. f. exp. Path.*, 1921, **91**, 46.

[5] Takahashi and Inoko, *Arch. f. exp. Path.*, 1890, **26**, 401.

[6] Ishiwara, *Arch. f. exp. Path.*, 1924, **103**, 209.

[7] Macht, *Trans. Am. Therap. Soc.*, 1940, **40**, 62.

Nine species of fish, the names of which are underscored in the table, were definitely toxic for growth of *Lupinus albus* seedlings. The eel, formerly known to be poisonous only in respect to its blood serum, is included among the toxic fishes. It was also found that muscle extracts of *Spheroides maculatus,* or balloon fish or puffer, and of the porcupine fish were similarly toxic for plant growth. The saline muscle extracts of a tenth specimen, the bur-fish *(Chilomycterus),* were also found to be very toxic for the plants. In addition to these, muscle extracts of the catfish, skate, shark (dogfish and hammerhead), and *Opsanus tau,* or toadfish, exhibited phytotoxic reactions. Of special interest were the results obtained with the beautiful little *Vomer setipinnis,* popularly known as the moonfish. Several of this species, obtained in Atlantic City, were found to be markedly toxic for plants. Whether the so-called stingarees or sting rays are truly poisonous fish or injure human beings by the wounds which they make with their jagged spikes and which afterward become infected has long been a mooted question. Results of

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[8] Macht, *Science,* 1930, **71**, 302.

[9] Macht, *Protoplasma,* 1937, **27**, 1.

TABLE I.
Toxicity of Fish Muscle Extracts.

| No. | Common Name | Scientific Name | Phytotoxic Index, % |
|---|---|---|---|
| 1 | "Alewife" | *Pomolobus pseudoharengus* | 82 |
| 2 | Banded drum | *Larimus fasciatus* | 80 |
| 3 | Black bass | *Micropterus dolomieu* | 80 |
| 4 | Black drum | *Pogonias cromis* | 105 |
| 5 | Bluefish | *Pomatomus saltatrix* | 80 |
| 6 | "Bonito" | *Auxis thazard* | 78 |
| 7 | Bowfin | *Amiatus calva* | 90 |
| 8 | Butterfish | *Poronotus triacanthus* | 81 |
| 9 | Carp | *Cyprinus carpio* | 90 |
| 10 | Catfish | *Ameiurus catus* | 48 |
| 11 | Channel bass | *Sciaenops ocellatus* | 80 |
| 12 | "Chub" | *Chaenobryttus gulosus* | 91 |
| 13 | Cod | *Gadus callarias* | 90 |
| 14 | Croaker | *Micropogon undulatus* | 90 |
| 15 | Eel | *Anguilla chrisypa* | 40 |
| 16 | Flounder | *Paralichthys dentatus* | 83 |
| 17 | Flying fish | *Prionotus carolinus* | 87 |
| 18 | Gambusia | | 89 |
| 19 | Goldfish | *Carassius auratus* | 88 |
| 20 | Haddock | *Melanogrommus aeglefinus* | 80 |
| 21 | Hake | *Urophycis regius* | 98 |
| 22 | Halibut | *Platysomatichthys hippoglossoides* | 82 |
| 23 | Herring | *Clupea harengus* | 100 |
| 24 | Kingfish | *Tarpon atlanticus* | 83 |
| 25 | Menhaden | *Brevoortia tyrannus* | 90 |
| 26 | Moonfish | *Vomer setipinnis* | 51 |
| 27 | "Mullet" | *Erimyzon sucetta* | 87 |
| 28 | Pike | *Esox americanus* | 98 |
| 29 | Pompano | *Trachinotus carolinus* | 110 |
| 30 | Porcupine fish | *Diodon hystrix* | 60 |
| 31 | Porgy | *Stenotomus chrysops* | 80 |
| 32 | Puffer | *Spheroides maculatus* | 51 |
| 33 | Rainbow trout | *Salmo irideus* | 81 |
| 34 | "Robin" | *Lepomis gibbosus* | 91 |
| 35 | Rock | *Roccus lineatus* | 100 |
| 36 | "Salmon trout" | *Cynoscion nebulosus* | 96 |
| 37 | Sand flounder | *Lophopsetta maculata* | 85 |
| 38 | Sand skate | *Pteroplatea maclura* | 59 |
| 39 | Smelt | *Menidia menidia* | 90 |
| 40 | Sea bass | *Centropristes striatus* | 103 |
| 41 | Shad | *Alosa sapidissima* | 100 |
| 42 | Shark (dogfish) | *Mustelus canis* | 62 |
| 43 | Silver squeteague | *Cynoscion nothus* | 84 |
| 44 | Spadefish | *Chaetodipterus faber* | 80 |
| 45 | Spanish mackerel | *Scomberomorus maculatus* | 98 |
| 46 | Spot | *Leiostomos xanthurus* | 80 |
| 47 | Stingaree | *Dasyatis say* | 46 |
| 48 | Sturgeon | *Acipenser oxyrhynchus* | 87 |
| 49 | Toadfish | *Opsanus tau* | 49 |
| 50 | Tuna, bluefin | *Thunnus thynnus* | 88 |
| 51 | Weakfish | *Cynoscion regalis* | 97 |
| 52 | White perch | *Pomoxis sparoides* | 81 |
| 53 | Whiting, Carolina | *Menticirrhus americanus* | 84 |
| 54 | Yellow perch | *Perca flavescens* | 87 |

this series of phytopharmacological experiments support the view that in addition to such trauma stingarees produce a poisonous substance demonstrable in their muscle juices.

All the muscle extracts which proved to be poisonous for seedlings of *Lupinus albus* were profoundly toxic for white mice on intraperitoneal injection. Their toxicity was indicated by marked depression of all the animals followed by convulsions in some but in most cases by death due to paralysis of the respiratory center. Extracts of all the other fishes listed in the table, however, had no such toxic effect on mice. Four mice were usually employed in each animal experiment to examine those extracts which had been found to be poisonous for plants while in making the phytopharmacological test 2 or 3 sets of experiments were carried out with at least 10 seedlings for each solution studied.

Of great scientific interest in connection with this work were the findings made with extracts from various species of *Tetraodontidae* and *Diodontidae,* from certain of which some years ago Japanese scientists extracted the so-called "fugu" poison. Their descriptions of the poisonous effects of this substance indicate that it is a neurotoxin, *i. e.,* a chemical exerting its effect chiefly upon the central nervous system, and many of the symptoms it produces are not unlike those effected by bites of various serpents. The present writers having been engaged in an intensive pharmacological, toxicological, and therapeutic study of snake venoms and particularly of cobra neurotoxin have begun a chemical and pharmacological investigation of extracts of some of the *Tetraodontidae* and *Diodontidae* in order to compare their properties with those of snake venoms.

It is to be noted that all experiments were made with fresh unheated suspensions. Exposure to heat destroyed the toxicity of most of the muscle extracts with the exception of those from the *Tetraodontidae* and *Diodontidae.* These could be heated to 60°C without markedly impairing their toxicity for plants. Prolonged boiling of all the extracts (5 to 10 minutes) causes disappearance of the poisonous properties of all the solutions, a finding which is true of most poisons of animal origin, including snake venoms. The venom of the cobra has been found to be more resistant to heat than that of other serpents so that it could be repeatedly heated to 60°C without being completely inactivated.[10]

The phytopharmacological and zoopharmacological data described are of considerable historical interest. It will be noted that with the exception of the catfish and eel, which are not ordinarily considered delicacies, all the "poisonous" fishes are inedible. Furthermore, all the fishes belonging to the toxic group underscored in the table possess either but rudimentary scales or none at all so that there seems to be some scientific basis for the ancient classification of edible and inedible fishes into two groups, namely, those which have scales and those which have not.[11]

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[10] Macht and Harden, *Am. J. Physiol.,* 1940, **129**, 411.

*Summary.* (1) Extracts of muscle tissue prepared with physiological saline at room temperature were obtained from 65 varieties of fish and examined pharmacologically. (2) It was found that extracts of muscle tissue obtained from ten different sets of fishes were poisonous for seedlings of *Lupinus albus* and for mice while suspensions of the other fifty-five specimens exhibited little or no toxicity. (3) A comparative study of the chemical and pharmacological properties of the poisonous fish muscle extracts with those of certain snake venoms is now in progress.

### 11949

## Effect of Repeated Administration of Delvinal Sodium [5-ethyl-5-(1-methyl-1-butenyl) barbituric acid] to Guinea Pigs.*

EMMETT B. CARMICHAEL AND WILLIAM D. THOMPSON.

*From the Department of Physiological Chemistry, School of Medicine, University of Alabama, University.*

Cope and Hancock[1] reported that the sodium salt of 5-ethyl-5-(1-methyl-1-butenyl) barbituric acid was an effective hypnotic with a high therapeutic ratio when administered either by stomach tube (oral) or by intraperitoneal injection. Hendrix[2] gave the same compound by stomach tube to both dogs and rats and stated that there was no indication of the development of tolerance to the drug in the case of dogs and that rats showed little or no tendency toward the development of tolerance.

In this paper, we wish to present the results of repeated intraperitoneal injections of delvinal sodium into 16 normal young guinea pigs.

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[11] Leviticus XI, 9-12.

* The delvinal sodium was kindly furnished by Sharp and Dohme, Inc., Philadelphia.

[1] Cope, A. C., and Hancock, E. M., *J. Am. Chem. Soc.*, 1939, **61**, 776.

[2] Hendrix, J. P., *J. Pharm. and Exp. Therap.*, 1940, **68**, 22.

The delvinal sodium was kept in a desiccator between weighings and a fresh aqueous solution (25 mg/1 cc) was prepared just previous to each series of injections. The dose (40 mg/kilo body weight) was selected, since it produced hypnosis that lasted about 3 hours in normal young guinea pigs. The drug was administered twice weekly, Tuesday and Saturday, by one of us (E.B.C.). As soon as the animals were recorded as asleep, they were placed in an incubator at about 90°F in order to avoid excessive loss of body heat and possible pneumonia. The injections were continued for 4 weeks. The guinea pigs were allowed dry hay, oats and water routinely up to the time of injecting the drug. Green vegetables were fed after each recovery from hypnosis and until about 15 hours previous to the administration of the drug.

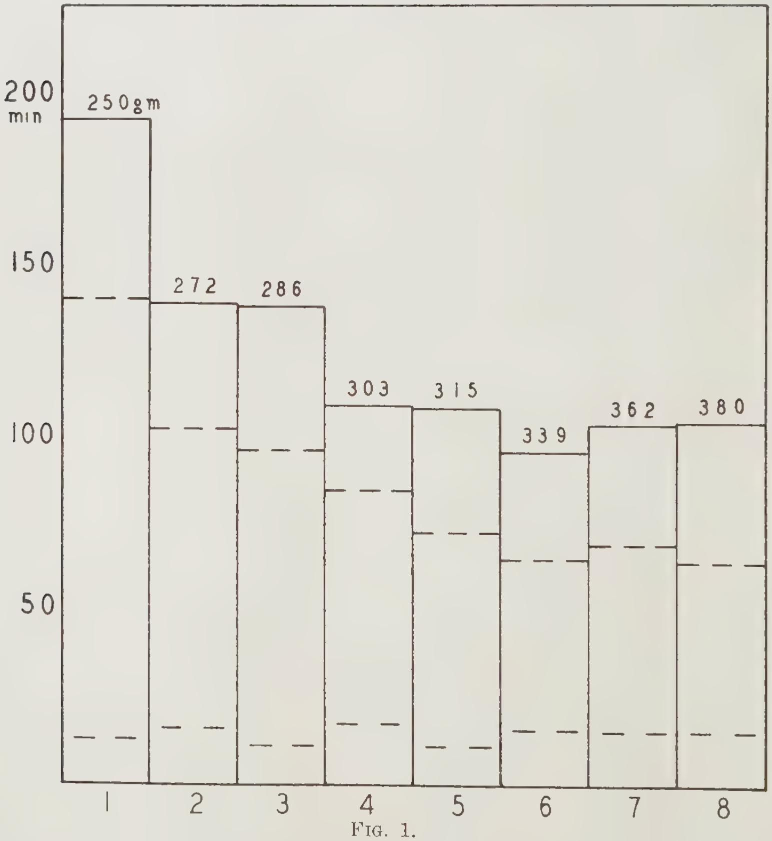

Fig. 1.

The graphs in Fig. 1 represent averages and each unit shows the results for a single day. The lower dotted line represents the time required to produce sleep and the upper dotted line represents the end of sleep. The average weight of the guinea pigs before each injection is given above the respective graph.

The periods of time required for the development of 3 stages in the hypnosis (anesthesia) were selected according to Carmichael and Posey[3, 4] in their studies with nembutal (pentobarbital sodium): (a) The time from the injection until the animal could not support and propel itself after stimulation by gently pulling the hair on the hips. This stage was considered the onset of sleep. (b) The time from the injection until the animal could be aroused sufficiently to walk forward after stimulation. This stage was considered the end of sleep. (c) The time from the injection until the animal could walk a distance of about a meter with a steady gait (without ataxia). This stage was considered the end of the hypnosis. Each animal was observed by this scheme after each injection and the data are illustrated in Fig. 1.

*Summary.* Guinea pigs were found to show a tolerance to delvinal sodium [5-ethyl-5-(1-methyl-1-butenyl) barbituric acid] on repeated administration of large doses of the drug. The average length of sleep dropped from 127.3 to 48.2 minutes following 8 semi-weekly injections of the drug, while the average length of hypnosis dropped from 191.9 to 104.1 minutes. The average weight of the guinea pigs increased from 250 to 380 g, which was more than a 50% increase in weight.

### 11950

## An A.C. Induction Flow Meter for Measurement of Blood Flow in Intact Blood Vessels.

A. Kolin. (Introduced by H. H. Sobotka.)

*From the Laboratories of the Mount Sinai Hospital, New York City.*

The principle of the electro-magnetic flow meter[2] is based on the fact that an electromotive force is induced in the blood as it flows through a magnetic field (the direction of flow forming preferably a right angle with the magnetic lines of force).* The induced e.m.f.

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[3] Carmichael, E. B., and Posey, L. C., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1329.

[4] Carmichael, E. B., and Posey, L. C., *Anesthesia and Analgesia*, 1937, **16**, 199.

* Further reference to the application of electro-magnetic induction in liquids may be found in Nos. 1, 3-9 of the bibliography appearing in this article.

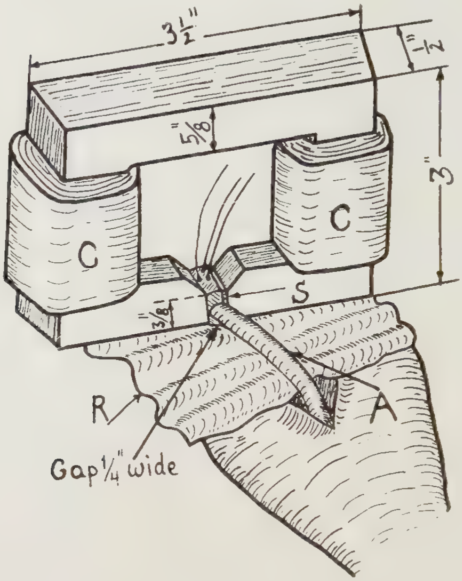

Fig. 1.

Diagram illustrating determination of blood flow in an exposed artery. C: Field coils of electro-magnet. S: bakelite sleeve. A: Artery. R: Dry rubber sheet insulating the exposed section of the artery from the adjacent tissues.

is linearly proportional to the velocity of flow. It is tapped by two electrodes placed at right angles to the magnetic lines of force and to the direction of flow. Owing to the electrical conductivity of the blood vessel wall the pick-up electrodes merely have to touch the outside of the vessel to establish a sufficient electrical contact with moving blood. Fig. 1 shows the perspective view of the arrangement and the dimensions of the magnet.

The modification presented here employs an electro-magnet fed by an alternating current. The use of an alternating magnetic field eliminates the disturbances due to polarization and variable galvanic potentials and makes it possible to use an a.c. amplifier. It introduces, however, the new difficulty of eliminating the a.c. voltage induced in the lead wires at zero flow. This interference has been abolished by the use of a special sleeve S and a compensating po-

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[1] Fabre, P., *C. R. Ac. Sc.*, 1932, **194**, 1097.

[2] Kolin, A., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 53.

[3] Kolin, A., *Am. J. Physiol.*, 1937, **119**, 355.

[4] Kolin, A., and Katz, L. N., *Ann. de Physiol. Physicochim. Biol.*, 1937, **13**, 1022.

[5] Katz, L. N., and Kolin, A., *Am. J. Physiol.*, 1938, **122**, 788.

[6] Wetterer, E., *Z. f. Biol.*, 1937, **98**, 26.

[7] Wetterer, E., *Z. f. Biol.*, 1938, **99**, 158.

[8] Jochim, K., *Am. J. Physiol.*, 1939, **126**, 547.

[9] Einhorn, H. D., *Proc. Roy. Soc. S. Africa*, 1940, **33**.

tentiometer P in conjunction with a filter F in the amplifying circuit. (Fig. 2.) The bakelite sleeve, S, contains a slit, Sl, which allows a blood vessel to slip into the round channel of the sleeve. This establishes the proper contact between the vessel wall and the electrodes, $E_1$ and $E_2$ (2/56 brass screws screwed into the bakelite flush with the channel wall). A coil, C, of 3 turns of copper wire is wound around the sleeve imbedded in the groove, G. The e.m.f. induced in this coil by the alternating magnetic field is applied to the terminals of the potentiometer, P, whose sliding contact is adjusted in order to cancel the undesired voltage induced at zero flow. This arrangement alone, however, is insufficient since it does

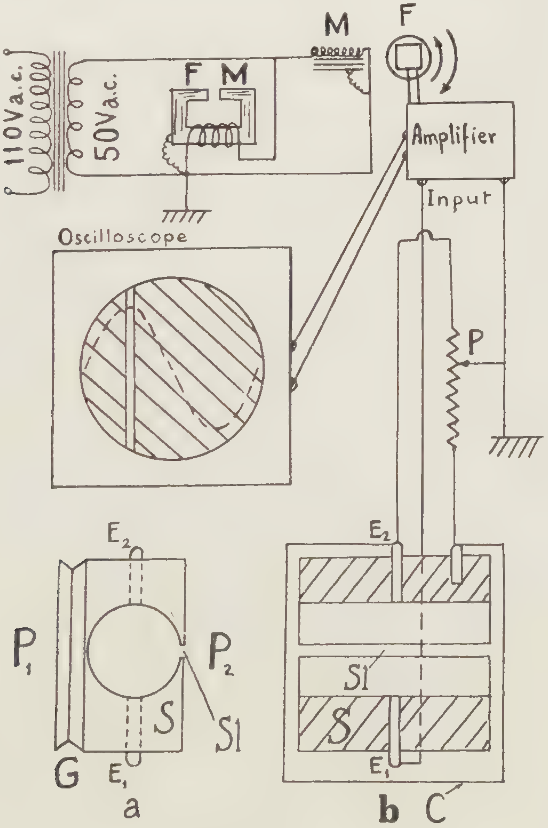

FIG. 2a.

Lateral view of the bakelite sleeve. $P_1$ and $P_2$ indicate the location of the magnet poles. S: Bakelite sleeve. Sl: Slit. G: Groove. $E_1$ and $E_2$: Pick up electrodes.

FIG. 2b.

Longitudinal section of the bakelite sleeve with the general flow-meter circuit. S: Sleeve. Sl: Slit. $E_1$ and $E_2$: Pick up electrodes. C: Coil (only one turn indicated instead of three). P: Potentiometer. F: Filter on turntable. M: Small magnet. FM: Flow-meter magnet.

not allow the phase of the compensating voltage to be varied. The variation of the phase is accomplished as follows: A No. 814-A General Radio amplifier is used in conjunction with the filter, No. 814-P3 (connected between the 2nd and 3rd stage). This filter, F, which passes a narrow frequency band around 60 cycles, is mounted on a turntable near a small laminated a.c. magnet, M, fed by the same transformer as the flow meter magnet, FM. A change of the orientation of the filter with respect to the magnet, M, results in a variation in magnitude of the voltage which is induced in the self-inductance of the filter. Reorientation of the filter with a simultaneous adjustment of the potentiometer, P, introduces in the amplifier output 2 alternating voltages of variable magnitude and a phase difference of 60 degrees. A combination of these voltages in suitable proportion allows a complete cancellation of the undesired voltage induced at zero flow.

The amplifier output voltage is applied to a cathode ray oscilloscope[†] with a horizontal sweep frequency of 60 cycles. The alternating voltage induced by flow produces a standing sine wave pattern, the maximum of which rises in linear proportion to the rate of flow and becomes inverted as the direction of flow is reversed. By screening off the oscillographic sine wave image[‡] except for a narrow slit at the location of the maximum only a fluorescent point remains visible, which indicates the rate as well as the direction of flow. It can be readily photographed. Since there is no lag in

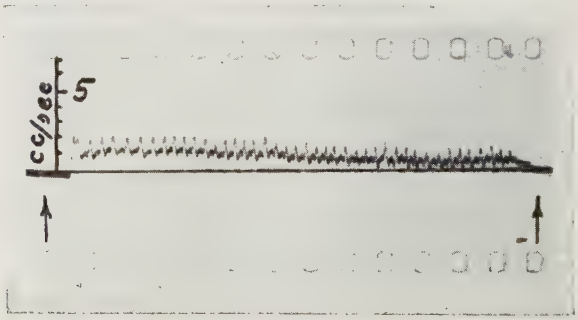

FIG. 3.

Excerpt from a tracing of blood flow through a dog's femoral artery following injection of pituitrin. (Film speed 8 cm/min.) Arrows indicate where blood flow was stopped by compressing the artery. The base line was drawn subsequently by connecting the horizontal sections obtained at zero flow. Amplification: 20% of the maximum gain.

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[†] We used an RCA oscilloscope type: TMV-122C with its amplifier at maximum gain.

[‡] Such a slotted mask can also be mounted in front of the photographic film which has the advantage of leaving the oscilloscope screen unobstructed.

induction of an e.m.f. the blood velocity during each phase of the cardiac cycle is instantaneously indicated. Fig. 3 shows a tracing of blood flow in an intact artery of a dog. The vessel was compressed from time to time to ascertain the location of the base line corresponding to zero flow. 0.1 cc/sec correspond at maximum amplification to a deflection of 1 mm so that a flow of .03 cc/sec still can be detected. The calibration is accomplished by perfusion of the excised blood vessel postmortem. The peak value of the magnetic field strength in the gap was 1500 Oersted. The entire magnet was coated with a layer of bakelite enamel, the pole pieces being particularly well insulated.

The method presented above has also been modified to permit the recording of flow in unexposed blood vessels *in situ*. A magnet with a gap large enough to enclose the neck of a medium-sized dog has been used. In order to measure the blood flow through the carotid artery, for instance, the sleeve is applied to the artery in the usual manner. The vessel is left in place, the lead wires are led off through the skin and the neck is closed. Thereafter one merely has to insert the neck between the large magnet poles to record blood flow. Further details on this modification of the method will be published elsewhere.

The author wishes to thank Dr. S. Feitelberg for his assistance in improving the photographic recording as well as for surgical help, and Drs. J. L. Weissberg and L. Gerber for their coöperation in extending the application of the method to unexposed blood vessels *in situ*.

## 11951 P

## Guinea Pig Copulatory Reflex in Response to Aqueous Extracts of Adrenal Cortex.

Olaf Torstveit and C. H. Mellish. (Introduced by R. K. Meyer.)

*From the Department of Physiology, The Lakeside Laboratories, Inc., Milwaukee, Wisconsin.*

It has been demonstrated that progesterone[1, 2] and desoxycorticosterone[3] will elicit the copulatory reflex in the spayed guinea pig

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[1] Dempsey, E. W., Hertz, R., and Young, W. C., *Am. J. Physiol.*, 1936, **116**, 201.

[2] Collins, V. J., Boling, J. L., Dempsey, E. W., and Young, W. C., *Endocrinology*, 1938, **23**, 188.

[3] van Heuverswyn, J., Collins, V. J., Williams, W. L., and Gardner, W. U., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 552.

TABLE I.
Effect of Cortin in Producing Copulatory Reflex in Guinea Pig.

| *Group | Dose in cc | % positive response |
|---|---|---|
| A | 0.5 | 36.7 |
| A | 1.0 | 55.0 |
| A | 1.5 | 81.7 |
| B | 0.5 | 36.4 |
| B | 1.0 | 54.5 |
| B | 1.5 | 72.7 |

*Groups A and B contained 60 and 11 pigs respectively.

after preliminary estrogen treatment but that certain androgens will not do so.[4]

We thought it of interest to determine whether aqueous extracts of the adrenal cortex which contain the life-maintaining principle would produce this reaction.

The method used to test the cortical extracts was similar to that described by other workers.[4] Two groups of guinea pigs, A and B, consisting of 60 and 11 animals respectively, were used. The results obtained from administering graded doses of the extract on successive weeks are shown in Table I.

It will be seen that there is a relation between the dose administered and the number of animals showing the copulatory reflex. The data on the small group are included since the response to each dose agrees so closely with that of the larger group. Furthermore, group B was given each dose of the extract one week prior to the administration of the corresponding dose to group A. Since these figures were tabulated, 30 animals representing one-half of group A have given a 50% response to a dose of 1 cc. This result offers further evidence of the reliability of the method.

The extract used in obtaining the data shown in the table contained 2.9 rat units[5] per cc. Other preparations which contained more than 2.9 rat units proved to be more active in producing the copulatory reflex. These preliminary findings indicate that there is a correlation between the life-maintaining and the copulatory-reflex activity of adrenal cortical extracts. It seems probable, therefore, that this reaction in the guinea pig might be used as a method for assaying such preparations.

Additional studies are being made of the relationship between the guinea pig copulatory-reflex response and other biological properties of adrenal cortical extracts.

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[4] Hertz, R., Meyer, R. K., and Spielman, M. A., *Endocrinology*, 1937, **21**, 533.

[5] Cartland, G. F., and Kuizenga, M. H., *Am. J. Physiol.*, 1936, **117**, 678.

## 11952

# Recognition of Sulfapyridine-Fast Pneumococci.

HANNAH Y. COTLER, MINNA T. KIRCHNER AND MARIE ROMANO.
(Introduced by R. S. Muckenfuss.)

*From the Bureau of Laboratories, New York City Department of Health.*

The regularity with which pneumococci become resistant to the action of sulfapyridine under experimental conditions *in vitro* and *in vivo*[1-4] and the occurrence of such strains in infections in man, have led to the development of a method for their recognition through the use of blood agar plates, which is described below. The results of its application to stock laboratory cultures, to specimens obtained from patients who responded poorly to treatment with sulfapyridine, and to specimens from pneumonia patients submitted for routine bacteriological examination indicate that this test is suitable for use in connection with the control of chemotherapy.

*Method.* Specimens from pneumonia patients, or subcultures from such specimens, are inoculated on 3 blood agar plates (citrated horse blood, 5% in nutrient agar) to 2 of which sulfapyridine is added sufficient to make concentrations of 5 mg % and 10 mg %. The third blood agar plate, without sulfapyridine, serves as a control. Equal sized loopfuls of material are used in order to make the inocula as nearly as possible the same on all 3 plates. The growth on the plates is compared after 24 hours in the incubator, and pneumococcus colonies on the control plate without sulfapyridine are identified as to type by capsule swelling reaction and as to bile solubility by spraying dried bile over the colony.[5] The susceptible pneumococcus on the plates containing sulfapyridine produces some partial (alpha type) hemolysis where the inoculum is heaviest, but separate colonies are rarely visible and of minute size. Stained preparations from hemolyzed areas show aberrant bacterial forms, and subcultures sometimes show viable pneumococci. Pneumococcus growth on the plates containing sulfapyridine is seldom obscured by overgrowth of other organisms as these are also markedly inhibited. The presence of a resistant pneumococcus growing freely on the sulfapyridine plates is readily recognized.

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[1] Ross, R. W., *Lancet*, 1939, **236**, 1207.

[2] MacLeod, C. M., and Daddi, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 69.

[3] Long, P. H., and Bliss, E. A., *Ann. Int. Med.*, 1939, **13**, 232.

[4] Mulder, J., van der Berg, R., and Eimers, G., *Ned. Tijd. v. Geneeskunde*, 1940, **84**, 923.

[5] Greey, P. H., *J. Inf. Dis.*, 1939, **64**, 206.

TABLE I.
Growth of Stock Pneumococcus Cultures on Blood Agar Plates with and without Sulfapyridine.

| Pneumococcus Type | Concentration of Sulfapyridine | | | |
|---|---|---|---|---|
| | None | 1mg% | 5mg% | 10mg% |
| 1 | Good | Good | Poor | Poor |
| 1 (Same as above but grown in broth containing increasing amounts of sulfapyridine) | ,, | ,, | Good | Good |
| 4 | ,, | Poor | Poor | Poor |

*Stock Laboratory Cultures.* The production of a sulfapyridine-resistant pneumococcus strain was undertaken in order to observe its growth on the differential media. A Type 1 pneumococcus culture, highly virulent for rabbits and mice and susceptible to the prophylactic action of sulfapyridine in both these animals, was grown in serum broth containing concentrations of the drug that were increased gradually from 2 mg % to 16 mg %. The resulting culture grew freely on blood agar plates containing as much as 16 mg % of sulfapyridine, whereas the growth of the parent strain was partially inhibited by a concentration of as little as 4 mg % of sulfapyridine. Table I shows the relative growth of the resistant strain and the parent susceptible strain, and for comparison the growth of a third strain, representing the greatest susceptibility found by these methods on blood agar plates containing various concentrations of sulfapyridine. The resistant culture was not changed perceptibly with regard to its type specificity or mouse virulence, nor in its susceptibility to the protective action of antipneumococcic serum.

Tests for growth on blood agar plates containing 1, 5 and 10 mg % of sulfapyridine have been carried out with 105 different strains, which include the 55 pneumococcal types described by Walter, *et al.,*[6] and 11 other types not yet completely studied. Some inhibition of growth of all strains occurred in the presence of 1 mg % sulfapyridine, although with 26 strains this was shown only by the smaller size of the colonies. Growth of all strains was inhibited to a marked degree by 5 and 10 mg % sulfapyridine.

*Cultures from pneumococcus infections unimproved by sulfapyridine therapy.* Brief summaries of diagnosis, outcome, therapy and bacteriological observations of 5 patients in whom the lack of therapeutic effect of sulfonamide drugs was particularly striking are given in Table II. The first 3 patients yielded cultures of sulfapyridine-resistant pneumococci late in the disease, after the patient had received comparatively large amounts of the drugs. Two of the pneumococcus strains, the Type 4 (case G.H.) and the Type 1 (case C.M.) grew freely on blood agar plates containing 10 mg % of sulfapyridine, while the Type 25 strain (case A.T.) grew freely in the presence of a concentration of 5 mg % of sulfapyridine, but not in a concentration of 10 mg %. The sulfapyridine-resistance shown by these 3 cultures, and that of the stock strains, made resistant *in vitro* are stable characteristics of the microörganisms and have persisted in culture media without sulfapyridine for over 3 months. In case C.M. a susceptible microörganism was recovered from a spinal fluid obtained the day before death and a resistant strain the day of death. Since 3 days intervened between the time of the spinal tap and the isolation of the pneumococcus from the fluid obtained on the day of death, the possibility that the resistance of this culture had developed in the specimen tube rather than the patient, is to be considered as a source of possible error.

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[6] Walter, A. W., Guevin, V. H., Beattie, M. W., Cotler, H. Y., and Bucca, H. B., *J. Immunol.,* in press.

*Application of culture methods to specimens submitted for bac-*

TABLE II.
Sulfapyridine Resistance of Pneumococci from Infections Unimproved by Sulfonamide Therapy.

| | | | | Sulfapyridine therapy | | | | Culture | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Before culture | | Total | | | | |
| Case | Diagnosis | Outcome Recovered or died | Days of disease | G | No. of days | G | No. of days | Days of disease | Pneumo. type | Reaction to sulfapyridine in blood agar plates |
| G.H. | Broncho pneumonia | Recov'd | 20 | 64 | 16 | 82 | 21 | 26 | 4 | Resistant |
| A.T.* | Meningitis Endocarditis | Died | 22 | 36 | 4 | 40 | 9 | 17 | 25 | ” |
| C.M.† | Meningitis<br>Bilateral Otitis Media (onset unknown) | ” | 3 | 15 | 2 | 17 | 3 | 2<br>3 | 1<br>1 | Susceptible<br>Resistant |
| F.D. | Lobar pneumonia | Recov'd | 14 | 43 | 9 | 43 | 9 | 26 | 18 | Susceptible |
| G.A. | Lobar pneumonia Endocarditis | Died | 21 | 35 | 7 | 96 | 12 | 16 | 25 | ” |

*Sulfanilamide 18 g in 2 days before sulfapyridine.
† ” 8 g ” 14 ” ” ”

*teriological diagnosis of pneumonia.* A consecutive series of 105 specimens received for examination in the Health Department, Pneumonia Control Division Laboratories, were cultured, using one plate containing sulfapyridine 10 mg % and one control without sulfapyridine, to determine whether sulfapyridine-resistance occurs regularly and frequently in such specimens. Eighty-three of the 105 specimens contained pneumococci. The susceptibility of 35 of these pneumococcus strains was determined within 24 hours by direct inoculation of the specimens on blood agar plates, and of 29 by the use of subcultures which required 48 hours or longer. Subcultures were not obtained from 19 specimens. The direct inoculation of specimens on blood agar plates was successful most regularly (24 of 37 specimens) when the specimen showed a type-specific pneumococcus by Neufeld examination.

All of the microörganisms obtained from the 64 fully tested specimens (56 patients) were susceptible to the bacteriostatic action of blood agar plates containing 10 mg % of sulfapyridine.

The property of resisting the bacteriostatic action of sulfapyridine comparable in degree to that produced experimentally may appear as a characteristic of pneumococci in human pneumococcus infections where therapeutic response does not occur. Such resistance may be recognized by the observation of the growth on blood agar plates containing sulfapyridine. The diagnostic procedure outlined is recommended for use in pneumococcus infections that do not respond satisfactorily to chemotherapy with sulfapyridine.

### 11953 P

## Changes of Arterial Blood Pressure and Renal Hemodynamics by Injection of Angiotonin in Human Beings.

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*From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Indiana.*

Renin, the so-called "renal pressor substance" is vaso-inactive unless permitted to interact with a pseudoglobulin present in blood plasma, which has tentatively been denominated "renin-activator."[1] The interaction of renin and renin-activator yields a crystallizable, dialyzable, thermostable product, angiotonin.[2] Intravenous injections of this substance in normal animals cause sharp pressor responses which diminish slowly with repeated injections. Nephrectomized animals are highly sensitive to the pressor action of this substance and do not show tachyphylaxis with repeated injections.[3] Slow intravenous infusions of angiotonin in dogs increase arterial pressure and cause renal vasoconstriction, predominantly of the glomerular efferent arterioles, but do not alter cutaneous temperatures.[4] The effects of angiotonin infusion therefore simulate those of renin,[5] although they are not as long sustained.

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[1] Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 214.

The present report is concerned with observations of blood pressure and renal hemodynamics during single injections and intravenous infusions of angiotonin into human beings. Electrocardiographic records and observations of venous pressure and skin temperature were obtained in some instances. These, together with further observations of renal functional changes will form part of a subsequent report.

Arterial pressure was determined by auscultation. Effective renal blood flow was calculated from the hematocrit ratio and apparent plasma diodrast clearance and is equal to approximately 90% of total renal blood flow.[6] Diodrast was determined in blood and urine by a modification of the method of White and Rolf[7] and inulin by the method of Corcoran and Page.[8] A mixture of diodrast, phenol red and inulin in 0.9% NaCl was given intravenously at a slow rate during the observations of renal clearances.[9]

The angiotonin solutions were prepared by the method of Helmer and Page.[2] The responses to single injections of angiotonin were observed only with reference to arterial pressure, while diodrast and inulin clearances were observed during the infusions of angiotonin. The infusions were preceded by a single dose, intravenously, of 1.0 to 1.5 cc of the angiotonin solution and continued by small injections of the solution into the rubber tubing which carried the diodrast-inulin infusion.

*Single Injections.* Single intravenous injections of from 0.5 to

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[2] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

[3] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 495.

[4] Corcoran, A. C., and Page, I. H., *Am. J. Physiol.* 1940, **130**, 335.

[5] Corcoran, A. C., and Page, I. H., *Am. J. Physiol.*, 1939, **126**, 354

[6] Corcoran, A. C., Smith, H. W., and Page, I. H., *Am. J. Physiol.*, 1940, **129**, 338.

[7] White, H. L., and Rolf, D., PROC. SOC. EXP. BIOL. AND MED., 1940, **41**, 1.

[8] Corcoran, A. C., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 601.

[9] Smith, H. W., Goldring, W., and Chasis, H., *J. Clin. Invest.*, 1938, **17**, 263.

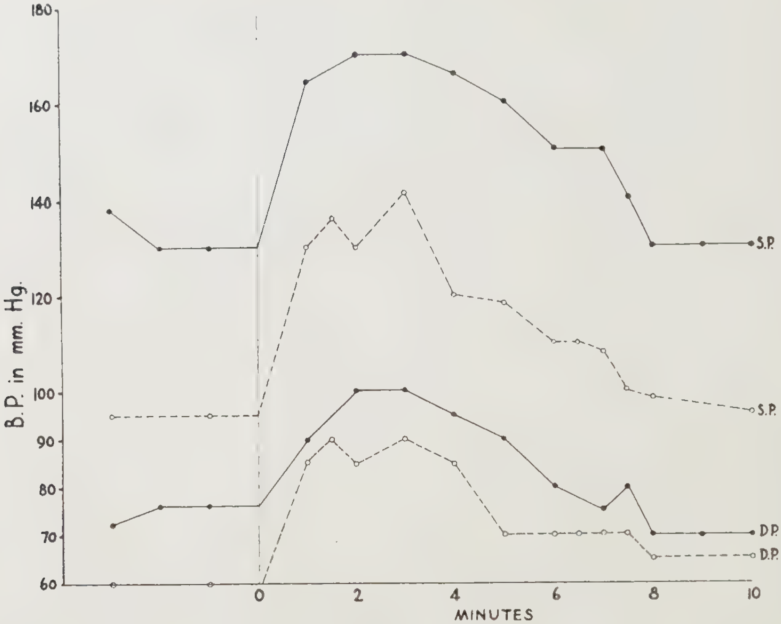

CHART 1.

Effects of single intravenous injections of angiotonin on arterial blood pressures in two patients. Patient 1. •——• Dose injected 0.6 cc. Patient 2. o- - o Dose injected 1.0 cc. Ordinate: Systolic (S.P.) and diastolic (D.P.). Blood pressure in mm Hg. Abscissa: time in minutes.

1.0 cc of angiotonin increase both systolic and diastolic arterial pressure. The arterial pressure returns to its normal value at the end of 6 to 9 minutes. The injections are not associated with subjective discomfort, nor do they cause pallor nor other outward vasomotor change. A graphic chart of 2 such injections (Chart 1) illustrates the typical response of normotensive patients.

*Angiotonin Infusion.* The graphic record of an experiment in which an initial intravenous dose of angiotonin was succeeded by a succession of smaller doses into the infusion fluid is shown in Chart 2. It will be noted that the increased arterial pressure is associated with decreased renal blood flow and with an increase of filtration fraction (ratio inulin/diodrast clearance) and that the pressor and renal responses subsided during the 19 minutes which followed the last addition of angiotonin to the infusing fluid.

*Comment.* The effects of intravenous injections and infusions of angiotonin in human beings closely resemble the effects of this substance in experimental animals. The increase of filtration fraction which occurs during angiotonin infusion is evidence of increased intraglomerular pressure[10] and, since it is associated with decreased

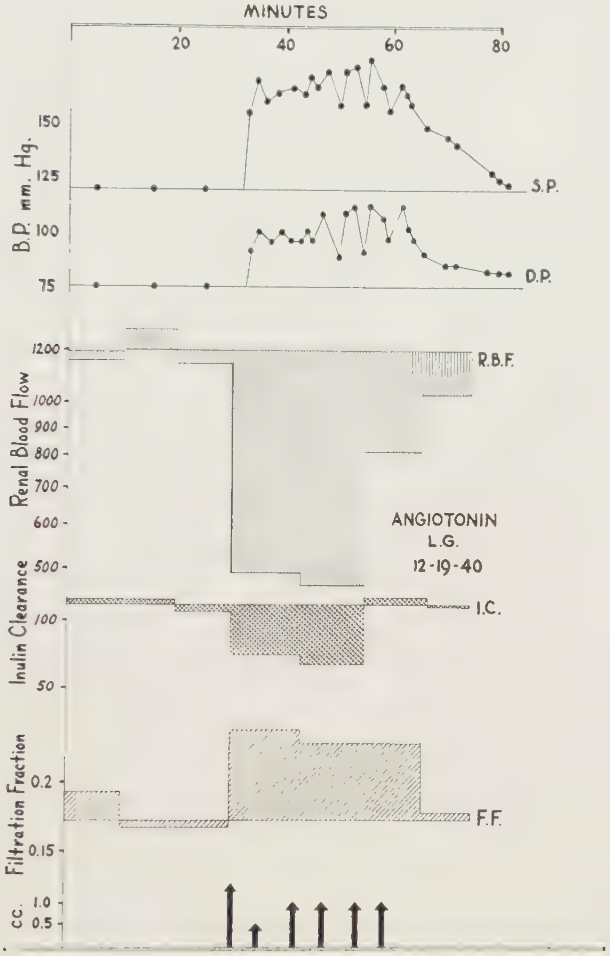

CHART 2.

Effects of infusion of angiotonin on arterial blood pressure, renal blood flow, inulin clearance and filtration fraction in patient L.G. Ordinates from above downwards: arterial systolic (S.P.) and diastolic (D.P.) blood pressures; effective renal blood flow (R.B.F.) in cc per min.; inulin clearance (I.C.) in cc per min.; filtration fraction (inulin/diodrast clearance ratio, F.F.); cc indicates volumes of initial intravenous injection of angiotonin and of subsequent injections into the infusing fluid. Effective renal blood flow, inulin clearance and filtration fraction are plotted semilogarithmically. Abscissa: time in minutes.

renal blood flow, is the characteristic result of constriction of the glomerular efferent arterioles. Efferent arteriolar constriction with absolutely or relatively decreased renal blood flow characterizes the kidney in hypertension in human beings.[11] Since the blood of

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[10] Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W., *J. Clin. Invest.*, 1938, **17**, 683.

[11] Smith, H. W., The Porter Lectures, Univ. of Kansas, 1939.

patients suffering from hypertension contains an excess of a vasoconstrictor which, like angiotonin, is potentiated in the presence of the blood of nephrectomized animals,[12] it is not unlikely that the increased arterial pressure and altered renal hemodynamics of hypertension may be the result of the unopposed action of angiotonin.

### 11954 P

## Relationship of Vitamin A Blood Level in the Rat to Vitamin A Intake and to Liver Storage.

J. M. LEWIS, O. BODANSKY, K. G. FALK AND G. MCGUIRE.

*From the Department of Pediatrics and the Department of Laboratories, Beth Israel Hospital, and the Laboratory of Industrial Hygiene, New York City.*

This study was undertaken to determine whether the concentration of vitamin A in the blood has any relationship to the vitamin A intake and to the storage in the liver. Groups of albino rats, 3 to 4 weeks of age, were fed amounts of vitamin A ranging from 0 to 1000 international units daily. The vitamin A supplement used for rats receiving 100 units or less daily was a reference cod liver oil obtained from the United States Pharmacopea Committee, whereas rats receiving 1000 units were given a vitamin A concentrate. After the rats received these preparations for 6 weeks, the vitamin A content of their bloods and livers was determined 24 hours after the last dose of the vitamin.

The method employed for the vitamin A determination of the blood and liver was based on the Price-Carr reaction. As the diet was devoid of carotene, this substance was not found in the blood or livers of the rats. The vitamin A in the blood was extracted directly with petroleum ether and alcohol as recommended by Clausen and McCoord,[1] whereas the liver was treated by a preliminary saponification. The extracts were evaporated in nitrogen, taken up with chloroform and then treated with antimony trichloride. The intensity of the resulting blue color was measured at 620 mμ with the use of an Evelyn Photoelectric Colorimeter.[2] The reading was translated into biological units (international) by re-

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[12] Page, I. H., *J. Exp. Med.*, 1940, **72**, 301.

[1] Clausen, S. W., and McCoord, A. B., *J. Pediatrics*, 1938, **13**, 635.

[2] Dann, W. J., and Evelyn, K. A., *Biochem. J.*, 1939, **32**, 1008; Kimble, M. S., *J. Lab. Clin. Med.*, 1939, **24**, 1055.

TABLE I.
Relationship of Vitamin A Blood Level in the Rat to Vitamin A Intake and to Liver Storage.

| No. of Vitamin A units given daily for 6 weeks | No. of Animals | Avg wt | | No. of Vitamin A units per 100 cc of blood plasma | | Avg No. of Vitamin A units per liver | Avg No. of Vitamin A units per gram of liver |
|---|---|---|---|---|---|---|---|
| | | At beginning of study, g | At end of 6 weeks, g | Avg | Range | | |
| 0 | 13 | 45 | 98 | 0 | — | 0 | 0 |
| 1 | 11 | 44 | 119 | 7 | 5-11 | 0 | 0 |
| 2 | 31 | 40 | 140 | 14 | 5-42 | 0 | 0 |
| 10 | 31 | 40 | 159 | 45 | 9-53 | 0 | 0 |
| 25 | 16 | 41 | 172 | 69 | 37-103 | 24 | 3 |
| 50 | 15 | 41 | 172 | 100 | 91-102 | 228 | 34 |
| 100 | 21 | 42 | 173 | 112 | 81-152 | 710 | 113 |
| 1000 | 26 | 43 | 177 | 110 | 68-142 | 10,000 | 1270 |

ferring to a calibration curve obtained with a sample of halibut liver oil of known biological potency.

The relationship of the vitamin A blood level to vitamin A intake and to liver storage may be noted in Table I. It will be observed that when the intake was less than 50 units daily the vitamin A concentration of the blood was directly related to the vitamin A intake. Thus, groups of rats receiving 0, 1, 2, 10, 25, and 50 units daily had average blood levels of 0, 7, 14, 35, 69, and 100 units, respectively, per 100 cc of blood plasma. Increasing the feeding above 50 units daily had no appreciable effect on the vitamin A level of the blood, the average value being 112 units for rats receiving 100 units daily and 110 units for those receiving 1000 units daily. Thus, maximal blood concentrations of vitamin A were obtained with an intake of 50 units daily. In this connection it should be added that McCoord and Luce-Clausen[3] found that supplementing the basal Sherman B diet with halibut liver oil did not affect the vitamin A concentration of the blood in rats. Since one-third of the Sherman B diet consists of dry milk, it therefore contains an appreciable quantity of vitamin A. It is possible, in view of our experiments, that the vitamin A intake of the rats receiving the basal Sherman B diet alone was 50 units or more daily and that adding extra quantities of vitamin A to this diet had, therefore, no effect on the blood level.

No vitamin A was detected in the livers of rats receiving 10 units or less daily. When the intake was 25 units or more, vitamin A was found in the liver, the amount stored being directly related to the intake. The concentration of the vitamin A in the liver, therefore may be compared with that in the blood; in the latter a maximal level was reached with an intake of 50 units, whereas in the liver the concentration continued to mount as the intake was increased.

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[3] McCoord, A. B., and Luce-Clausen, E. M., *J. Nutrition,* 1934, **7**, 557.

Blood concentrations of less than 37 units per 100 cc of plasma were associated with no liver storage; those between 37 and 53 units were accompanied by little or no liver storage; whereas blood levels above 53 units were always associated with vitamin A in the liver, the amount stored, however, varying considerably and having no relation to the absolute blood level. These findings therefore indicate that the blood level may be of value in ascertaining whether there is any storage of vitamin A in the liver, the chief reservoir of this vitamin in the body. Further experiments concerning the relationship of blood level to storage are being carried out and will be reported at a later date.

We wish to express our appreciation to Mr. Bertrand Flusser and to Miss Mildred Weinstock for their coöperation in this study.

## 11955 P

## Specific Cause and Nature of Ulcerative Enteritis of Quail.*

CHARLES C. BASS.

*From the Laboratory of Clinical Medicine, School of Medicine, Tulane University of Louisiana, New Orleans.*

In a former communication[1] I have identified a previously unrecognized, Gram-negative, anaerobic bacillus as the specific cause of ulcerative enteritis of quail (so-called "quail disease"). I also gave details of the technic by which the organism may be demonstrated in suitable material, directed attention to the chronic carrier as a factor in maintaining and spreading the infection and recorded observations indicating transmission of the infection to young quail chicks through eggs laid by carriers.

The purposes of the present communication are: (a) to correct and contradict a statement in the previous paper relative to spore formation, (b) to give technic for isolation of the organism in pure culture, (c) to call attention to coprophagy as an important source of transmission in captive quail, and (d) to record successful immunization of birds by vaccination with killed cultures of the specific organism.

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* Studies on which this report is based have been aided by donations from Mr. A. B. Freeman and Mr. C. S. Williams of New Orleans, and by a grant from the Special Research Fund of the Tulane University of Louisiana.

[1] Bass, Charles C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 377.

(a) The statement previously made that "occasionally spores can be found on slide preparations made direct from lesions" has been found to be incorrect. Examination of hundreds of specimens made from pure cultures and from lesions has failed to show spores. Reëxamination of some of the original specimens on which the first statement was based reveals that what were taken for spores were something else, therefore contradicting the previous statement relative to spores.

(b) Pending development of differential culture media and cultural technic, and possibly more favorable culture media than is now available, attention is called to a medium and technic which are entirely satisfactory for isolation and maintenance of pure cultures of the organism This medium is the thioglycollate medium of J. H. Brewer,[2] which I modify slightly by the addition of 0.1% agar for semisolid cultures or 1% for solid cultures. The composition of the Brewer medium is as follows:†

| Infusion from 37.5 g pork per 100 cc. | |
|---|---|
| Peptone | 1.0% |
| Dextrose | 1.0 |
| Sodium chloride | 0.5 |
| Sodium thioglycollate | 0.1 |
| Agar | 0.05 |
| Methylene blue (1 to 500,000) | 0.0002 |

The medium is tubed and autoclaved in the usual manner. The sodium thioglycollate establishes and maintains a state of anaerobiosis except at the surface which is exposed to the air and where partial aerobic condition is indicated by a green color imparted by the methylene blue.

The medium keeps well at room temperature for a month or two. It permits cultivation of strict anaerobes, such as the quail disease bacillus seems to be, without the aid of seals, special tubes or other complicated equipment.

It is difficult to secure material from intestinal lesions that is not contaminated with other bacteria which greatly increase the difficulty of isolating pure cultures from such material. On the other hand, lesions in the liver (which are found in more than 10% of diseased birds) usually have only the specific bacillus.

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[2] Brewer, J. H., *J. Bact.*, 1940, **39**, 10.

† A powdered concentrate of the solids of this medium can be obtained from Baltimore Biological Laboratory, Baltimore, Md. To prepare, it is only necessary to dissolve the proper amount of the powder (and for our purpose, the additional agar) in distilled water, dispense in tubes and autoclave.

The necrotic plug in a very small lesion should be removed with teasing needles and inoculated directly into the medium. Good growth occurs after 24 to 48 hours' incubation and the pure culture can be maintained indefinitely if transplanted at least every 3 or 4 days. Cultures in the semi-solid medium should be transferred with capillary pipettes instead of the platinum loop.

(c) Quail disease can be transmitted by forced feeding of fresh droppings from diseased birds. On the other hand, dry or old droppings are innocuous. I have often observed coprophagy in birds crowded together in small pens. Several quail breeders have confirmed this observation from their own experience. It is likely that coprophilia, or unnatural tendency to eat feces, develops in such birds as a result of dietary or environmental faults or deficiencies associated with close confinement.

I have penned clean birds with carriers and have repeatedly observed that transmission often occurs if the pen is so constructed and arranged as to favor accessibility of their droppings to the birds. On the other hand, many times I have kept infected and susceptible birds together in the same pen without spread of the infection, provided the pen is so constructed as to prevent exposure of the droppings to the birds.

The disease spreads in cages with wire bottoms made of hardware cloth with spaces one-third inch or less, but does not if the mesh is one-half inch or larger; provided there is no other opportunity for droppings to be accessible to the birds.

When wide distribution has occurred in a quail stock, it takes from 6 to 10 months for all infected birds to die out and the disease to disappear, even under the above indicated precautions.

(d) Vaccination with killed cultures of the quail disease bacillus produces a high degree of immunity against experimental inoculation with live cultures. Exact dosage and methods have not been satisfactorily established. However, I have obtained adequate protection by giving to half grown birds, 2 intramuscular injections at 5-day intervals, of 0.5 cc vaccine made from 24- to 36-hour cultures in the above described semi-solid medium and killed with merthiolate, 1 to 10,000. Birds thus immunized survive feeding of several hundred times the infectious (and usually fatal) dose for non-immune birds.

## 11956

# Maintenance of Adrenalectomized Guinea Pigs.*

WILLIAM G. CLARK. (Introduced by F. H. Scott.)

*From the Department of Zoology, University of Minnesota, Minneapolis.*

Schachter and Bebee[1] have described a technic for adrenalectomizing guinea pigs, and have found this animal extremely sensitive to the absence of the adrenal cortex and very responsive to cortical extract, dying of adrenal insufficiency in 3 to 4 days after maintenance for 10 to 12 days on cortical extract.

During some other investigations on adrenalectomized guinea pigs, a few observations were made on the dietary requirements of normal and adrenalectomized guinea pigs, responsiveness to cortical hormone and salt therapy, and survival, which were thought worthwhile to report here.

The technic of adrenalectomy was somewhat similar to that of Schachter and Bebee, being performed in 2 stages. The right adrenal was removed first, 2 weeks elapsing before the left was removed. From experiments scattered from 1938 through 1940, over 40 animals were adrenalectomized. In 20 guinea pigs, the average survival after removal from hormone or salt therapy was 4.6 days average (extremes 2 to 10, depending upon age).

In some preliminary work on dietary requirements, several different rations were examined. These are listed in Table I.

The animals were individually caged and fed and watered daily from glass finger bowls.

*Ration No. 1.* Nine male guinea pigs, average body weight 612 g, were fed ration 1 and tap water. In 6 days, the smallest animal of the group had a swollen abdomen and some alopecia. In 10 days, one animal developed diarrhea and died on the 12th day. Two others were very weak on the 12th day. The average weight loss of the group was 57 g in this time. The calculated ascorbic acid intake was 20 to 40 mg daily per guinea pig.

*Ration No. 2.* Seven males, average body weight 680 g, were fed ration 2 and tap water for 30 days, at which time the average weight loss was 18 g, the losses being much greater for younger animals.

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* This study was made possible by grants from the American Academy of Arts and Sciences, and from the American Association for the Advancement of Science.

[1] Schachter, R. J., and Bebee, M. O., Jr., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 541.

TABLE I.
Experimental Diets for Guinea Pigs.

| Item | Ration No. | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Dextrinized starch (Corn Products) | — | — | 55 | — | 50 |
| Dextrose (Corn sugar) | 55 | 55 | — | 56 | — |
| Casein | 20 | 20 | 18 | 18 | 20 |
| "Celluflour" | 10 | 10 | — | — | — |
| Lard | — | — | — | — | 7 |
| Soybean oil (Archer-Daniel) | 8 | 8 | 8 | 7 | — |
| Corn oil ("Mazola") | — | — | — | — | 9 |
| Wheatgerm oil (cold pressed) | 2 | 2 | 2 | 2 | — |
| Salt mixture (1) | 2 | 2 | 2 | 2 | 2 |
| Yeast concentrate (2) | 3 | 3 | 3 | 3 | — |
| Dried brewer's yeast (Fleischman) | — | — | — | — | 10 |
| Ascorbic acid (3) | 0.1 | 0.1 | 0.2 | 0.2 | — |
| Oleum percomorphum (Mead-Johnson) | 0.001 | 0.001 | — | — | — |
| Codliver oil (USP) | — | — | 2 | 2 | 2 |
| Leaf-ground alfalfa (fresh dried) | — | 10%* | — | — | — |
| Dehydrated, pulverized grass (4) | — | — | 10 | 10 | 20%* |

(1) Hubbell, Mendel, and Wakeman.[2]
(2) Upjohn. Dry powder concentrate.
(3) Cryst. Supplied by Chas. Pfizer and Co., Inc., New York.
(4) Canned under nitrogen. Supplied by Cerophyll Laboratories, Kansas City, Mo.
*Signifies that the item comprised 10% and 20% of rations 2 and 5 respectively.

*Ration No. 3.* Twelve males, average body weight 562 g, were fed ration 3 for 9 days. After 8 days, 4 animals were near collapse, and the average weight loss was 113 g. On the 9th day, 2 died in convulsions.

*Ration No. 4.* Ten males, average body weight 510 g, were fed this diet for 30 days. Two animals refused to eat and were discarded. One animal developed pneumonia, lost weight, and was discarded. Daily food consumption averaged 30 to 50 g. The group gained an average of 118 g in the 30 days. Hence this diet, containing 10% dehydrated grass, was fairly adequate for this period of time.

*Ration No. 5.* This ration has been tried on several animals at various times, and found to be somewhat superior to ration 4 for animals of all ages. As an example, 5 animals, average body weight 800 g, gained 85 to 130 g in 40 days on ration 5, containing 20% dehydrated grass.

These observations are in accord with those of Cannon and Emerson,[3] Kohler, Randle, Elvehjem, and Hart,[4] and Kohler, Randle, and Wagner[5] (*cf.* also Kohler and Schnabe[6]) which show that guinea pigs require a water-soluble factor obtained from grass juice in addition to the known B-complex and ascorbic acid, dying in 3 to 10 weeks if it is not supplied.

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[2] Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition,* 1937, **14**, 273.

[3] Cannon, M. D., and Emerson, G. A., *J. Nutrition,* 1939, **18**, 155.

[4] Kohler, G. O., Randle, S. B., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 154.

Fourteen guinea pigs, average body weight 498 g (420-585), were adrenalectomized in 2 stages, as described above. After removal of the first adrenal, they were fed ration 4. Five died in 19-30 days, with symptoms of a respiratory infection. All the others steadily gained weight for 30 days, when the second adrenal was removed. Seven unilaterally adrenalectomized guinea pigs served as controls. Desoxycorticosterone acetate in oil† in 0.5 to 1.0 mg doses was administered subcutaneously daily to the doubly adrenalectomized animals. Nine or 10 days after the second adrenal was removed, the average body weight of all animals, including the controls began to fall, and after 10 more days, had decreased 75 to 100 g. It is possible that the grass juice factor content of the grass powder had decreased in this time, as it had been kept in bottles in the refrigerator for some time. At this point, 10% freshly opened dehydrated grass was mixed with 90% ration 4, thus increasing the level of the grass juice factor. The controls gained 25 to 85 g in 6 days, whereas the doubly adrenalectomized animals continued to lose (25 to 70 g) in the same period of time. These results indicated that a diet adequate for normal guinea pigs and containing an excess of the grass juice factor, still failed to maintain adrenalectomized guinea pigs. The grass powder contained 4.65% K and 0.57% Na. Ration 4 contained 0.80% K, thus with 90% ration 4 and 10% additional grass powder, the K content increased to 1.2%, a 0.4% increase. It is doubtful that this much increase would be very detrimental when desoxycorticosterone acetate was being administered.

After this period, all animals were fed Purina "complete rabbit chow", whole oats, and fresh greens (lettuce and cabbage) daily, with the hormone therapy continued as before. On this high potassium diet, the doubly adrenalectomized animals gained an average of 145 g over a 26-day period, as compared with 40 g in the controls. Two animals in both groups died during this time, with symptoms of pneumonia, and showed heavy infiltration of macrophages in spleen imprints. Hormone therapy was then withdrawn from the remaining 7 animals in the doubly adrenalectomized group to test for possible hypertrophy of adrenal fragments or accessory bodies. In 5 days, 3 animals fell sharply in weight, the other 4 larger animals maintaining weight for 7 to 8 days before decreasing. One mg of desoxycorticosterone acetate prevented further weight loss. Hormone therapy was again discontinued, and the tap water was replaced by 1% NaCl, 0.2% $NaHCO_3$ drinking fluid. The 7 experimental guinea pigs increased in weight from an average of 629 to 758 g, a gain of 129 g in 41 days, as compared with an increase of 159 g in the controls. Thus salt intake replacing the desoxycorticosterone acetate acted for at least 6 weeks as a complete replacement therapy on the ration used.

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[5] Kohler, G. O., Randle, S. B., and Wagner, J. R., *J. Biol. Chem.*, 1939, **128**, lv.

[6] Kohler, G. O., and Schnabe, C. F., Am. Chem. Soc., 99th Meeting, 1940.

† Supplied by Roche-Organon, Inc.

To further test for hypertrophy of possible adrenal fragments or accessory bodies, the salt solution was then again replaced by tap water. Four of the 7 animals lost an average of 25 g in 6 days, while the larger animal maintained weight during this time. Salt was then resupplied, and 0.5 cc Wilson cortical extract administered daily for 3 days. The animals were fed ration 5. Two of them did not recover the weight loss suffered during the salt removal test, and died in 6 and 7 days. The remaining 5 guinea pigs gained an average of 107 g after 34 days on the new regime, and when salt solution was again replaced with tap water, they promptly died, surviving 4.6 days. They had lost an average of 92 g in this time. These 5 animals had been maintained on various dietary regimes and on salt and hormone therapies for over 5 months following adrenalectomy. In all probability, since these animals grew at a normal rate when compared with the control animals for a 40-day period in which they received salt drinking fluid but no cortical hormone, they might have been maintained for weeks at a time by such methods. Also, since 0.5-1.0 mg desoxycorticosterone acetate daily maintained adrenalectomized guinea pigs weighing 600 to 1000 g in a normal condition, as compared with 1.0 mg which is required by the immature rat,[7] and since the animals do not necessitate the use of low potassium-high sodium diets like adrenalectomized dogs, the guinea pig offers an extremely sensitive and inexpensive animal for investigations involving the adrenal cortex.

*Summary.* Several diets were tested for adequacy in the nutrition of the guinea pig, and the necessity of the grass juice factor for this animal was confirmed. A diet high in the grass juice factor and adequate for normal guinea pigs, failed to maintain adrenalectomized guinea pigs given amounts of desoxycorticosterone acetate which were adequate when a normal diet was fed. Adrenalectomized guinea

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[7] Grollman, A., *J. Pharm. and Exp. Therap.*, 1939, **67**, 257.

pigs can be maintained for at least 6 weeks on a normal diet if salt drinking fluid is offered.

Desoxycorticosterone acetate subcutaneously administered in daily doses of 0.5 to 1.0 mg produces normal growth in adrenalectomized guinea pigs.

Even though adrenalectomized guinea pigs had been maintained for over 5 months by salt and hormone therapy, they died on an average of 4.6 days after discontinuing this therapy.

## 11957

## Effect of Obstructive Jaundice on Polyarthritis in Rats.*

Jane E. Snow and Harry M. Hines.

*From the Department of Physiology, Cornell University Medical College, New York City.*

Hench[1] reported that marked and generally complete remissions from various rheumatic symptoms occur in patients with the onset of spontaneous jaundice. Later[2] he reported further confirmatory observations and stressed the fact that the phenomenon seemed to depend upon the severity of jaundice as measured by the concentration of serum bilirubin rather than upon the type of jaundice.

Because of these clinical observations it seemed of interest to investigate the effect of obstructive jaundice on arthritis in an experimental animal. Collier[3] first described a spontaneous polyarthritis in rats. Findlay, Mackenzie, MacCallum and Klieneberger[4] reported a somewhat similar disease in rats and were able to cultivate a pleuropneumonia-like organism from affected joints which, upon inoculation into rats, reproduced the disease. A culture of Findlay's organism, designated $L_7$, was obtained.

*Methods.* Young albino rats (average age, 6 weeks; weight, 100 g) were used. For culturing the $L_7$ organisms 20% horse serum nutrient broth was employed, 48-hour cultures showing distinct turbidity or growth. The size of the infective dose of

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* This work was conducted with support of funds generously given by the late W. E. Benjamin to Dr. John Staige Davis, Jr., for studies on arthritis.

[1] Hench, P. S., *Proc. Staff Meet., Mayo Clinic,* 1933, **8**, 430.

[2] Hench, P. S., *Arch. Int. Med.,* 1938, **61**, 451.

[3] Collier, W. A., *Geneesk. Tijdschr. Ned.-Ind.,* 1938, **78**, 2845.

[4] Findlay, G. M., Mackenzie, R. D., MacCallum, F. O., and Klieneberger, E., *Lancet,* 1939, **2**, 7.

organisms was determined by the individual body weight of the animal (0.3 cc 48-hour culture per 50 g of body weight). Injections were made by the intravenous route and littermate controls were simultaneously injected for each series of experimental animals. The first symptoms of developing arthritis appeared in the control animals approximately 48 hours after injection. Ligation of the common bile duct of the experimental animals was performed under anesthesia at intervals before and after the injection of the organisms. Serum bilirubin determinations were made on several of the experimental animals at various intervals after duct ligation. Since Tang and coworkers[5] have reported the bile-soluble property of pleuropneumonia organisms, it seemed of interest to determine whether jaundiced rat blood would affect the growth of $L_7$ organisms. Pour plates with a concentration of 20% whole blood in nutrient agar were made. It was found that blood removed from rats 2 to 12 days after bile duct ligation inhibited the growth of $L_7$ organisms, whereas normal rat blood supported abundant growth. It also seemed advisable later in the course of the work to use an organism which is not bile-soluble for an additional series of experimental animals. For this purpose a streptococcus of the "matt" variety, Group A, was obtained, which produced arthritis in rats.† The inoculating dose was 0.3 cc 24-hour broth culture per 50 g body weight. The resulting arthritis was not as severe as that induced by the $L_7$ organisms which, in contrast to the streptococci, were passed serially in rats at frequent intervals in order to maintain a maximum virulence.

The extent of arthritic involvement was estimated by the number of joints involved and the degree of tenderness and oedema. The knee and ankle joints were most frequently affected, with the wrist, then tarsal and carpal interphalangeal joints next in frequency.

*Results.* In Table I the degree of arthritic involvement is recorded as: 1+, 1 or 2 joints tender without noticeable oedema; 2+, 2 or 3 joints involved with slight oedema; 3+, 3 or 4 joints involved with moderate to marked oedema; 4+, animal incapacitated with 5 or more joints showing marked oedema. The table indicates the time of bile duct ligation with respect to the injection of infective organisms. It shows the time of onset and progressive degree of arthritic involvement up to 12 days about which time the control animals

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[5] Tang, F. F., Wei, H., McWirter, D. L., and Edgar, J., *J. Path. Bact.*, 1935, **40**, 391.

† This organism was obtained from Dr. Sidney Rothbard of the Department of Pathology, Cornell University Medical College, New York City.

TABLE I.

| No. of rats | Series | Time of duct ligation with respect to injection of organisms | Avg degree of arthritic involvement days after injection | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 8 | 10 | 12 |
| 3 | I | 6 days prior to injection of $L_7$ | 0 | 0 | 0 | 0 | 1+ | 2+ | 2+ | 2+ | 2+ |
| 3 | | Non-ligated controls | 0 | 1+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 3+ |
| 3 | II | 24 hr prior to injection of $L_7$ | 0 | 0 | 0 | 1+ | 2+ | 2+ | 2+ | 2+ | 2+ |
| 3 | | Non-ligated controls | 0 | 1+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 2 | III | 20 hr after injection of $L_7$ | 0 | 0 | 0 | 1+ | 2+ | 2+ | 2+ | 2+ | 2+ |
| 2 | | Non-ligated controls | 0 | 1+ | 2+ | 4+ | 4+ | 4+ | 4+ | 3+ | 3+ |
| 2 | IV | 48 hr after injection of $L_7$ | 0 | 1+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 3+ |
| 2 | | Non-ligated controls | 0 | 1+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 3+ |
| 3 | V | 6 days after injection of $L_7$ | 0 | 1+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 3+ |
| 3 | | Non-ligated controls | 0 | 1+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 3+ |
| 3 | VI | 24 hr prior to injection of Strep. | 0 | 1+ | 2+ | 3+ | 3+ | 3+ | 3+ | 2+ | 2+ |
| 3 | | Non-ligated controls | 0 | 1+ | 2+ | 3+ | 3+ | 3+ | 3+ | 2+ | 2+ |
| Avg conc. of bilirubin per 100 cc blood | | | | | | | | | | | |
| Days after ligation | | | | 0 | 1 | 3 | | 6 | 9 | | 12 |
| Mg | | | | 1 | 6.3 | 8.8 | | 13.4 | 10.2 | | 9.9 |

usually begin to show spontaneous recovery and the ligated animals begin to show the adverse effects of jaundice. The average concentration of serum bilirubin as determined on several of the experimental animals at intervals after bile duct ligation is also recorded.

*Summary.* Ligation of the common bile duct of rats prior to or 20 hours after the injection of $L_7$ organisms delayed the onset of arthritis, and the subsequent degree of joint involvement up to 12 days' time was markedly diminished. However, when duct ligation was performed after the onset of arthritis there was no effect upon the course of the disease. Since rat arthritis induced by the injection of hemolytic streptococci was not influenced by bile duct ligation, it seems probable that the effect of obstructive jaundice on the development and severity of $L_7$ arthritis in rats was due to the bile solubility of the $L_7$ organisms.

## 11958

# An Improved Cannula for Gastric and Intestinal Fistulas.

J. EARL THOMAS.

*From the Department of Physiology, The Jefferson Medical College of Philadelphia.*

The care of dogs with cannulated gastric or intestinal fistulas is often difficult because many of the animals will pull their cannulas out at the first opportunity. In the latter part of the year 1938 we undertook to design a cannula which could not be pulled out. To accomplish this it was necessary to use a larger inner flange than could conveniently be installed when the cannula was constructed in the usual way. The cannula was, therefore, designed with a removable flange which could be inserted separately into the stomach or intestine; the remainder of the cannula could then be attached to it. Once the flange is inserted, no great force can be applied to it through the visceral wall because of danger of injuring the mucosa; consequently, it was necessary to provide a wrench which could be applied through the lumen for tightening the flange on the cannula. The cannula and the wrench are illustrated in Fig. 1.

To install the cannula, the viscus to be cannulated (stomach or intestine) is secured through an abdominal incision and an opening made, barely large enough to admit the separated flange. After the flange has been inserted, the appropriate end of the inner tubular portion of the cannula is inserted through the opening and screwed into the flange, as far as possible, by hand. The wrench is then assembled inside the cannula in such a way that the hooks on the lower ends of the ⅛ inch rods grasp the flange in its narrow diameter. The tapered plug with a knurled head is slid down the ⅜ inch rod and rotated until the fins which project from its sides fall into the grooves on the inner aspect of the cannula; the screw joint is then firmly tightened. After this portion of the cannula has been tied in place with a purse-string suture, the inner tube is brought out through a stab wound in the lateral abdominal wall and the outer metal part screwed on from the outside. Generous lubrication of the cannula with sterile petrolatum facilitates this operation. After healing is complete the animals require no more attention than stock animals.

Since the new type cannula was devised, it has been used in preparing gastric and duodenal fistulas in 9 dogs, all of which are living at the present time except one, which lived for 18 months

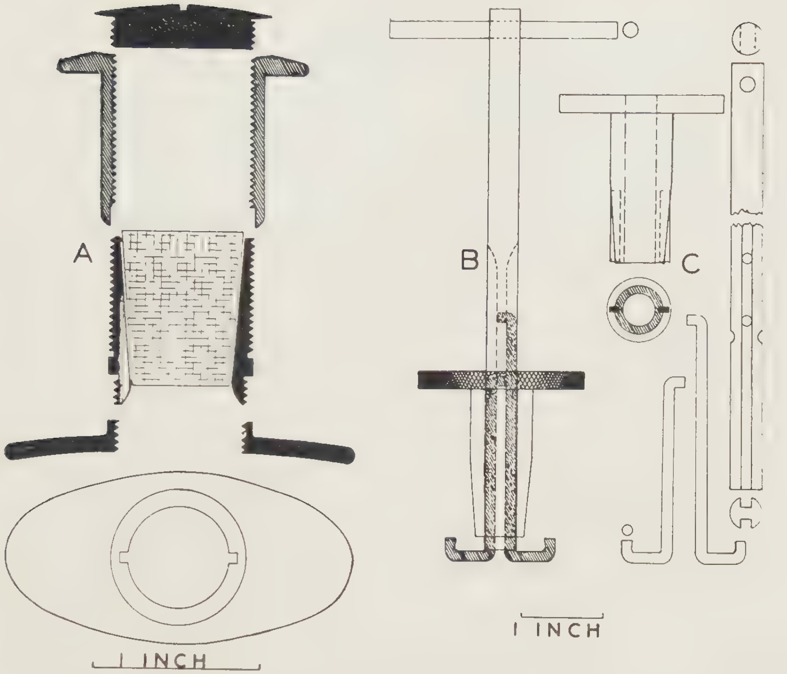

FIG. 1.

Apparatus Described in the Text.

A. Sectional view of the parts of the cannula shown disassembled. At the bottom the flange is shown in outline with the inner tubular portion screwed in place. The inner flange and tube and the cap (black) are made of hard rubber, the outer portion (diagonal shading) of aluminum alloy "17ST." The stopper is a No. 6 cork. The rubber parts of the cannula are sterilized in alcohol, since they cannot be boiled without warping.

B. The flange wrench, assembled.

C. Parts of the flange wrench shown disassembled. The material is stainless steel, except the tapered plug with knurled head which is made of aluminum alloy "17ST."

Note that the scale for "B" and "C" is one-half that for "A."

after operation. This is in contrast with our previous experience over a period of 8 years, in which approximately half our animals were lost within the first few weeks after operation, generally because the cannulas either came out or were pulled out or destroyed by the animal.

### 11959

# Production of Uterine Tumors in the Guinea Pig by Local Implantation of Estrogen Pellets.

W. H. Perloff* and R. Kurzrok.

*From the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, and Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University.*

Observers have long noted a relationship between ovarian activity and uterine fibromyomatous tumors in the human, and recently several workers have been able to produce similar growths in the uteri of guinea pigs.[1] The latter has been accomplished by the regular subcutaneous injection of large amounts of estrogenic substances over long periods of time. (18% of their injected animals showed uterine fibroids.) We were interested in determining whether the implantation of crystalline estrogen locally in the uterus would give rise to fibromyomatous growths in a higher percentage of instances than has been found with subcutaneous administered estrogen. Pellets of *estradiol benzoate,* each weighing approximately 3 mg were implanted in the uterine horn of each of 12 adult virgin female guinea pigs. This was done by carefully raising the peritoneum from the uterine muscle and placing the pellet into the pocket thus formed. A black silk suture served both to close the pocket and act as a marker. In 6 of these guinea pigs small pellets of cork or paraffin were introduced in a similar manner into the opposite horn. These animals were left undisturbed for 32 to 150 days and were then sacrificed.

In 3 other pigs a black silk suture was employed to close empty pockets and an injection of estradiol benzoate of 10,000 R.U. was administered subcutaneously every 20 days for a total of 60,000 R.U. In 2 additional animals we implanted 3 mg pellets of *estrone* into the uterus, and in 5 pigs 3 mg pellets of testosterone proprionate. Finally, in 2 animals a pellet of *estradiol benzoate* was placed in one horn of the uterus and a testosterone propionate pellet in the other. All of these animals were kept under observation for 150 days and then sacrificed.

*Results.* Seven unoperated controls were normal. In the 12 guinea pigs in which estradiol benzoate pellets were implanted into one horn of the uterus, either alone or with a paraffin or cork control in the second horn, fibromyomatous tumors were found surrounding the estrogenic pellet in 9 cases. No tumors were found around the cork or paraffin implants. Hydrometria were observed in 6 of the animals and were associated with hydrosalpinx in 2 cases. Pyometria alone were noted in 2 pigs and were associated with hydrometria in 2 others. Fibromyomatous tumors were therefore observed in 75% of the animals.

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* Ciba Fellow in Endocrinology.

[1] Nelson, Warren O., *Endocrinology*, 1939, **24**, 50.

The 3 pigs injected with estradiol benzoate subcutaneously in which silk sutures were introduced into the uterine peritoneum showed no masses. The 2 animals in which estrone pellets were implanted showed no tumors and the 2 animals in which estrogenic and androgenic pellets were implanted showed tumors only at the sites of the implantation of the estrogenic pellets.

Microscopically, the firm gray tumors were composed of varying amounts of muscular, fibrous, and collagenous tissues. The smaller ones contained a large proportion of muscle fibers but the larger ones consisted predominantly of fibrous tissue. In many scattered areas throughout the entire uterus, fibrous tissue replacement of uterine muscle was noted, particularly near the implanted estrogenic pellet. Many of these areas had associated with them collagenous deposition. The collagen was found particularly at the periphery of the fibrous tissue and appeared to be the first step in the replacement of muscle by fibrous tissue. Other microscopic changes resembled those previously described by many workers. The endometrial glands were cystic, showing widespread degeneration and necrosis leading in several cases to pyometria. The cervical epithelium was hyperplastic showing increased keratinization and down-

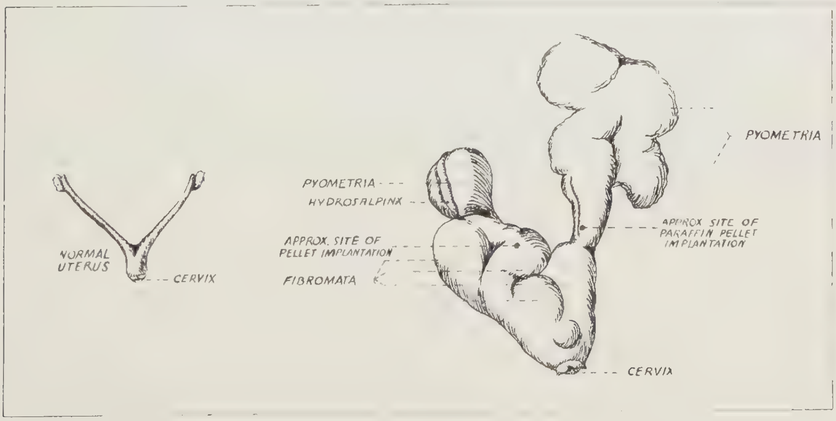
NORMAL UTERUS
CERVIX
PYOMETRIA
HYDROSALPINX
APPROX. SITE OF PELLET IMPLANTATION
FIBROMATA
PYOMETRIA
APPROX SITE OF PARAFFIN PELLET IMPLANTATION
CERVIX

Fig. 1.

Life size drawing of the uterus before implantation and the same uterus 150 days after the implantation of 3 mg of estradiol benzoate.

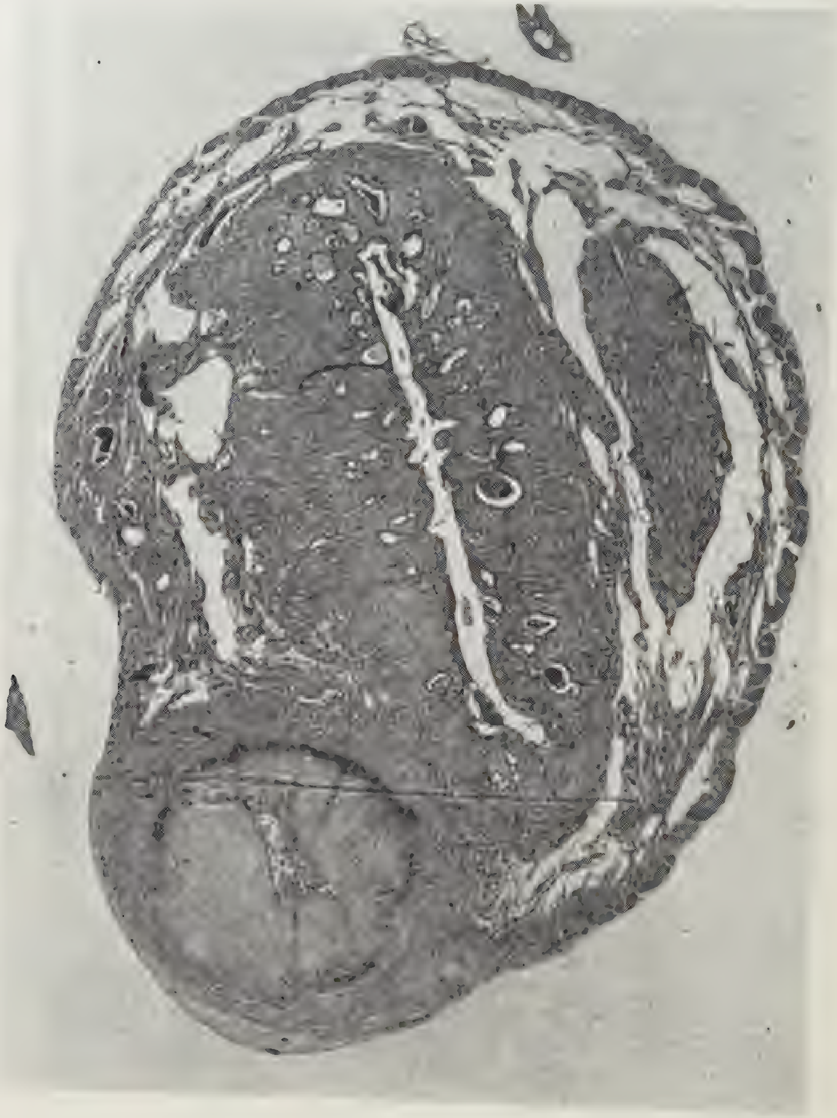

FIG. 2.

This section (low power) was taken so as to include a small tumor and the adjacent uterus.

growths into the submucosa with pearl formation. The vagina was also markedly cornified with thickening of the prickle cell layer and penetration of epithelial cords into the submucosa and pearl formation.

*Summary.* Small pellets of estradiol benzoate implanted locally into the uterus produced fibromyomatous tumors in 9 out of 12 guinea pigs (75%) after intervals varying from 32 to 150 days. Estrone in the same concentration did not produce tumors in 2 animals. From the findings made in 2 additional animals, it appears that androgen may have a tendency to limit the extent to which the estrogen is able to produce these tumors.

We wish to thank Miss Jane Smelser and Miss Anne Hammet for preparing the histological material and Mr. George Smelser for his advice and assistance.

We are indebted to Prof. B. P. Watson and to Dr. Carl Bachman for their help and criticism in preparing this material, and to Mr. Fred Roseberry for his careful drawings. The hormones used in these experiments were furnished by the courtesy of Mr. Robert Mautner of the Ciba Pharmaceutical Products, Incorporated.

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### 11960 P

## Androsterone Effect on Pituitary and Mammary Gland.*

R. P. REECE.

*From the Department of Dairy Husbandry, New Jersey Agricultural Experiment Station.*

Two estrogens, estrone and estradiol benzoate,[1] and one androgen, testosterone propionate,[2] have been shown to augment the lactogen content of the rat pituitary gland. These substances are likewise capable of inducing mammary gland growth. The administration of androsterone,[3, 4] however, has produced no detectable growth of the rat mammary gland. It was, therefore, of much interest to determine the influence of androsterone on the lactogen content of the pituitary gland.

Thirty sexually mature rats were ovariectomized and paired on the basis of body weight. One of each pair was injected subcutaneously daily for 15 days with 200 $\gamma$ of androsterone.[†] The other one of each pair received no treatment. Control and experimental animals were sacrificed on the 16th day of the experiment. Mammary glands from both groups were removed and studied as whole mounts. The pituitary glands were removed, weighed, and assayed for their lactogen content by injecting the suspended tissue intradermally over the crop glands of common pigeons. One-sixth of a control pituitary gland was injected (divided into 4 daily doses) over the right crop glands of 2 pigeons and one-sixth over the left crop glands of 2 additional pigeons. An equal amount of experimental pituitary tissue was injected over the left crop glands of the first pair of pigeons and one-sixth over the right crop glands of the second pair of pigeons.

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* Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

[1] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 266.

[2] Reece, R. P., and Mixner, J. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 66.

[3] Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

[4] Nelson, W. O., and Merckel, C. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 823.

The mean difference of the individual comparisons of the weights of the pituitary glands was not significant. The lactogen assay results revealed that 8 of the pituitaries from the injected rats contained more lactogen than did the glands from control rats, 2 were equal to, and 5 contained less lactogen. The mean difference of the lactogen content of the pituitary glands was not significant. At the time of sacrifice the mean difference of the body weights was significant, the control animals weighing more than the injected animals. The mammary glands of the control and experimental rats were similar in appearance, involutionary changes being observed in both groups of glands.

*Conclusions.* The daily administration of 200 $\gamma$ of androsterone for 15 days into sexually mature spayed rats caused no significant change either in pituitary weight or pituitary lactogen content and produced no detectable growth of the mammary glands.

### 11961 P

## Use of Helium for Determination of Pulmonary Capacity.

GEORGE R. MENEELY AND NOLAN L. KALTREIDER. (Introduced by Wm. S. McCann.)

*From the Department of Medicine, School of Medicine and Dentistry, University of Rochester, and the Medical Clinics of the Strong Memorial and Rochester Municipal Hospitals, Rochester, New York.*

Hydrogen has been used for the determination of the pulmonary capacity by VanSlyke and Binger,[1] who pointed out the danger of explosion and of poisoning with arsine. This procedure was replaced by oxygen dilution methods, particularly the Christie method,[3] which has been in use in this laboratory for the last 10 years. A recent report[2] has resulted in new interest in hydrogen, because of the ease and rapidity of analysis for this gas by thermal conductivity. A preliminary report is made at this time of a new method employing helium, because it has all the advantages and none of the dangers of the hydrogen method.

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[†] The androsterone used in this study was kindly supplied by Dr. E. Schwenk of the Schering Corporation.

A closed spirometer system with a blower is used. Carbon dioxide is absorbed by soda-lime. An important precaution is the addition of a water bottle to replace water vapor lost in the soda-lime. Dead space is measured by the oxygen dilution method. Part of the mixture circulating in the respiratory system is shunted through a thermal conductivity cell* with precautions to assure complete saturation of the gas mixture with water vapor. A meter, which is connected to the cell, is calibrated for helium in air fully saturated with water vapor over a range of 0-14% and also for variation in oxygen concentration of the mixture from 18-23%. Seven hundred ml of 98.2% pure "Medical" helium is added from a graduated cylinder with a leveling bottle to 4,800 ml of air in the system. When this is mixed by the blower, the concentration is 12.5%. The meter reading is compared with the calibration curve to assure proper function of the indicating unit. Two hundred ml of oxygen are then introduced to approximate the concentration of air.

The subject, who has rested for one half hour in the position in which the determination is to be carried out, is now connected without regard to the phase of respiration he is in. At the same time, oxygen is allowed to enter the apparatus at a rate equal to the oxygen consumption of the subject. A respiratory tracing is taken during the seven-minute rebreathing period. At the end of this period the subject is disconnected, and the oxygen flow turned off. The circulation of gases in the closed system is continued until carbon dioxide absorption is complete. The location of the respiratory tracing with reference to the spirometer scale, the meter reading, and the temperature are all recorded.

Gain or loss of oxygen will be evident from the tracing. The meter reading is corrected for this change in oxygen concentration and the final percentage of helium read from the calibration curve. The calculation follows that for McMichael's hydrogen method: If X + B is a volume given by the equation:

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[1] Van Slyke, D. D., and Binger, C. A. L., *J. Exp. Med.*, 1923, **37**.

[2] McMichael, J., *Clinical Science*, 1939, **4**, 167.

[3] Christie, R. V., *J. Clin. Invest.*, 1932, **11**, 1099.

* Cambridge Instrument Company.

$$X + B = \frac{\text{Initial quantity of helium}}{\text{Final concentration of helium}}$$

and if B is the volume of the spirometer system at the end of a normal expiration, then X is the volume of the lung at mid-capacity.

Several corrections must be applied to X. About 10 ml of helium is taken up by the body in the 7-minute period, equivalent to an apparent increase of 110 ml in a pulmonary capacity of 2 liters. The dead space of the mouthpiece and valve was 38 ml. It may be calculated that there is an apparent increase of 30 ml in the capacity because of the difference in inert gas concentration between the lung and spirometer, if one assumes average values of 0.8 for the R.Q. and 2 liters for the mid-capacity. Very little error is introduced by subtracting the rounded total of these 3 figures from X found as above. The correction for temperature and vapor pressure differences is made in the usual way.

The volume at mid-capacity of the lungs of 5 normal subjects was determined by the Christie method,[3] by Lassen, Cournand, and Richards' modification of the Christie method,[4] and by the helium method with the same spirometer system and in duplicate. The error for the 3 methods was calculated and corrected for errors of distribution due to the small size of the sample at a level of significance of $P = 0.05$.[5, 6] It was found to be ± 0.18 liters, ± 0.22 liters, and ± 0.14 liters respectively. The helium figure averaged 292 ml less than the Christie method, and 202 ml lower than the modified Christie method. This represented a mean decrease of 14.1% and 10.2% respectively, with a probability between 0.98 and 0.99 that these means were different from zero.[6]

The great advantage of a procedure employing a gas, for which analysis by the thermal conductivity method is accurate, lies in the ease and rapidity with which analyses can be made. The advantage of using helium instead of hydrogen is the elimination of the dangers of explosion and arsenic poisoning. Because of the accurate location of the breathing level, the closed method is preferred to open methods. The high diffusibility of helium should result in greater accuracy in determining mid-capacity in subjects with pulmonary emphysema or obstruction, since it is more likely to be uniformly distributed in the lung. There should be less disturbance of normal pulmonary function with this method than with others, because the oxygen concentration of the respiratory mixture is atmospheric.

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[4] Lassen, H. C. A., Cournand, A., and Richards, D. W., Jr., *J. Clin. Invest.*, 1937, **16**, 1.

[5] Mainland, D., *The Treatment of Clinical and Laboratory Data*, Oliver and Boyd, London, 1938, p. 324.

[6] Fisher, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 1938, 7th Ed., Chap. V.

The method should not be regarded as fully developed. Further consideration must be given to the quantity of helium taken up during the rebreathing, to the correction for oxygen concentration, and to the true relation of spirometer helium concentration to alveolar helium concentration.

### 11962 P

## Effect of Glutathione on Tyrosinase and the Significance of the Dopa Reaction.*

Frank H. J. Figge.†

*From the Department of Anatomy, Yale University School of Medicine, New Haven, Conn., and the University of Maryland School of Medicine, Baltimore, Md.*

Bloch,[1] as a result of his discovery of the "dopa reaction" and because it had been impossible to demonstrate tyrosinase in the skin of any of the slightly pigmented vertebrates, postulated the existence of an enzyme that accelerated the oxidation of dihydroxphenylalanine. He named it "dopa oxidase", and claimed that this enzyme was responsible for melanin formation in human skin. Such an enzyme has not been isolated and its existence has been questioned many times. The controversy in regard to this has been discussed in comprehensive reviews.[2, 3]

Recently it has been shown[4] that the activity of the enzyme tyrosinase is regulated by the oxidation-reduction potential of substances

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* Aided by a grant from the American Association for the Advancement of Science.

† Rockefeller Foundation Fellow, 1940-1941.

[1] Bloch, Br., *Z. f. physiol. Chem.*, 1916, **98**, 226.

[2] Franke, W., in von Euler's *Chemie der Enzyme*, 1934, part 2, p. 38, J. F. Bergmann, München.

[3] Oppenheimer, *Die Fermente und ihre Wirkung*, 5th ed., 1926, vol. 2 and supplement, 1939, George Thieme, Leipzig.

[4] Figge, F. H., *J. Cell. and Comp. Physiol.*, 1940, **15**, 233.

added to the substrate. It was evident from this that both the enzyme tyrosinase and its substrate tyrosine could be present in a cell without melanin formation taking place if a sufficiently strong inhibitor such as reduced glutathione was also present. With this in mind, an investigation was started to determine whether the enzyme responsible for the "dopa reaction" was a separate and specific dopa oxidase or tyrosinase associated with an inhibitor. To investigate this possibility chemically the rates of melanin formation were determined in the following series of reaction mixtures:

1. Buffered dopa alone.
2. Buffered dopa plus various reversibly oxidizable substances.
3. Buffered dopa plus tyrosinase.
4. Buffered dopa plus tyrosinase and glutathione.
5. Buffered tyrosine plus tyrosinase.
6. Buffered tyrosine plus tyrosinase and glutathione.

Series 1 was included to study the rate of non-enzymatic oxidation in air. Series 2 was included to see if any of the reversibly oxidizable substances would accelerate or inhibit oxidation in air. The last 4 series were included to determine and compare the effect of glutathione on the rate of enzymatic oxidation of dopa and tyrosine. The pH of the substrate and concentrations of substances are indicated in the tables. The enzyme preparation was filtered potato juice. The methods used were essentially as described in previous papers.[4]

When the rates of melanin formation in Series 1 and 2 were compared, it was found that almost all of the reversibly oxidizable substances accelerated the oxidation of dopa by atmospheric oxygen (Table I). However, cysteine and reduced glutathione inhibited this oxidation slightly. The following substances accelerated the

TABLE I.
Influence of Reversibly Oxidizable Substances on Rate of Oxidation of $2 \times 10^{-3}$M Dihydroxyphenylalanine by Atmospheric Oxygen at pH 7.1.

| Series No. | Substances added $10^{-4}$M | Melanin concentration as indicated by percentage light absorption at end of 50 min. | 140 min. | 360 min. | 480 min. |
|---|---|---|---|---|---|
| 1 | Control | 0.0 | 1.5 | 13.5 | 28.5 |
| 2 | Potassium ferricyanide | 4.6 | 22.0 | 46.5 | 57.5 |
| | Phenol indophenol | 10.5 | 28.5 | 46.5 | 54.0 |
| | Toluylene blue | 6.0 | 29.0 | 48.0 | 54.0 |
| | Thionine | 9.0 | 39.0 | 60.0 | 65.0 |
| | Methylene blue | 5.8 | 37.0 | 61.0 | 68.0 |
| | Indigo disulphonate | 2.0 | 10.0 | 30.0 | 42.0 |
| | Phenosafranine | 1.4 | 8.0 | 26.0 | 36.0 |
| | Cysteine | 0.0 | 0.0 | 0.0 | 9.0 |
| | Glutathione | 0.0 | 0.0 | 0.0 | 3.0 |

non-enzymatic oxidation: potassium ferricyanide, phenol indophenol, toluylene blue, thionine, methylene blue, indigo disulphonate, and pheno safranin. The optimum rate of oxidation shifted during the experiment. At the end of the first period, the optimum rate was found in the phenol indophenol; by the end of the second period, this had shifted to thionine; and finally optimal oxidation of dopa to melanin was observed in the methylene blue mixture. The amount of melanin formed in this mixture was approximately 3 times that in the control. The acceleration of oxidation by thionine was almost as great as that of methylene blue. At present it is thought that this acceleration of oxidation by these reversibly oxidizable systems depends on their ability to accelerate hydrogen transport from the substrate molecules to atmospheric and dissolved oxygen, by acting as carriers in a short chain reaction.

The rates of melanin formation in Series 3 and 4, 5 and 6 respectively were compared (Table II). Small amounts of glutathione (0.1 mg-1.0 mg per 20 cc substrate) definitely inhibited the enzymatic oxidation of tyrosine, but inhibited the rate of oxidation of dopa by tyrosinase only very slightly.

This work does not prove conclusively that Bloch's postulated dopa oxidase does not exist, but it opens the way for at least two more interpretations of a positive dopa reaction not considered by Bloch, namely,

TABLE II.
Effect of Glutathione on Rate of Tyrosinase Oxidation of Tyrosine and Dihydroxyphenylalanine at pH 7.4.

| Series No. | Substrate 10–3M | Enzyme, cc | Glutathione in mg | Melanin concentration as indicated by percentage light absorption at end of | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 hr | 2 hr | 4 hr | 7 hr | 8 hr |
| | Tyrosine | .0 | .0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | " | .2 | .0 | 5.0 | 7.0 | 17.5 | 29.0 | 33.8 |
| | " | .2 | .0 | 4.5 | 7.5 | 17.0 | 29.0 | 33.0 |
| 4 | " | .2 | .1 | 4.0 | 5.0 | 12.0 | 24.0 | 26.5 |
| | " | .2 | .3 | 3.5 | 4.0 | 7.0 | 16.0 | 19.0 |
| | " | .2 | 1.0 | 2.5 | 3.0 | 6.2 | 6.0 | 7.5 |
| | Dopa | .0 | .0 | 2.0 | 3.7 | 13.0 | 28.0 | 34.0 |
| 5 | " | .2 | .0 | 51.0 | 73.5 | 86.0 | 89.0 | 90.0 |
| | " | .2 | .0 | 51.0 | 73.5 | 86.0 | 89.0 | 90.0 |
| 6 | " | .2 | .1 | 54.6 | 73.8 | 86.0 | 88.0 | 88.4 |
| | " | .2 | .3 | 54.0 | 72.0 | 84.5 | 86.5 | 87.2 |
| | " | .2 | 1.0 | 54.0 | 68.5 | 79.6 | 82.0 | 82.8 |

1. The presence of reversibly oxidizable substances in melanoblasts that accelerate oxidation of dopa by atmospheric and dissolved oxygen.

2. The presence of tyrosinase in melanoblasts along with an inhibitor. Such melanoblasts could oxidize dopa in air rapidly, but tyrosine would be oxidized so slowly as to be imperceptible over a short period of time because of the inhibitor.

That a positive dopa reaction indicates the presence of inhibited tyrosinase seems most likely and experiments are in progress to test this hypothesis.

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### 11963

## Electron Microscopic Studies of Biological Reactions. I. Reduction of Potassium Tellurite by *Corynebacterium diphtheriae.*

HARRY E. MORTON AND THOMAS F. ANDERSON.* (Introduced by Stuart Mudd.)

*From the Department of Bacteriology, University of Pennsylvania, School of Medicine, Philadelphia, Pa., and the RCA Research Laboratories, Camden, N. J.*

Since the beginning of the science of bacteriology, investigators have pursued the study of the chemical reactions brought about by bacteria. As a result there has accumulated a tremendous mass of knowledge concerning the types of reactions induced by bacteria, and the particular reactions which characterize various species. However, in all this work it has not been shown definitely whether the reactions in question take place exclusively on the cell surface, as some investigators maintain, or inside the cell, or both.

With a powerful new tool, the electron microscope[1] now available, it was thought desirable to attempt to determine the site of some chemical reaction brought about by a bacterial cell. Since the electron microscope detects variations in density, such a reaction should involve as products large particles or crystals resolvable with the microscope; *i. e.*, the particles must have diameters of at least 50 Å (5 m$\mu$). Another possibility would be to select for study reactions which involve substances which are capable of adsorbing particles or substances such as large protein molecules, organic compounds, or inorganic salts, any of which would give rise to a region of high density within or upon the cell. The addition of material to a suspension would, however, give rise to the possibility of the production of artifacts. We have consequently chosen for initial study a reaction of the first type, one brought about by an organism in a medium on which it produces an insoluble substance.

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* RCA Fellow of the National Research Council.

[1] Marton, L., *Phys. Rev.*, 1940, **58**, 57; *J. Bacteriology*, in press; and other references quoted in these papers; Zworykin, V. K., Hillier, J., and Vance, A. W., *Electrical Engineering*, in press.

The reaction chosen for study was discovered in 1900 by Klett.[2] He observed that many microörganisms, including the diphtheria bacillus, when grown in or upon culture media containing tellurite or selenite salts, reduces them to the free metals. Conradi and Troch[3] proposed a medium containing a tellurite salt for an aid in the diagnosis of *Corynebacterium diphtheriae* because on this medium the colonies of this organism have a characteristic black color due to the reduced tellurium, and because the medium exerts a selective bacteriostatic effect, allowing the diphtheria bacillus to grow but inhibiting certain other microörganisms. Although Klett observed gray particles inside and outside the cells of *Bacillus anthracis* grown on tellurite media, it is not known where the reaction occurs in the case of *C. diphtheriae*.

We have obtained micrographs with the RCA electron microscope of cells from diphtherial colonies growing on blood extract agar and on potassium tellurite chocolate agar. Fig. 1 shows un-

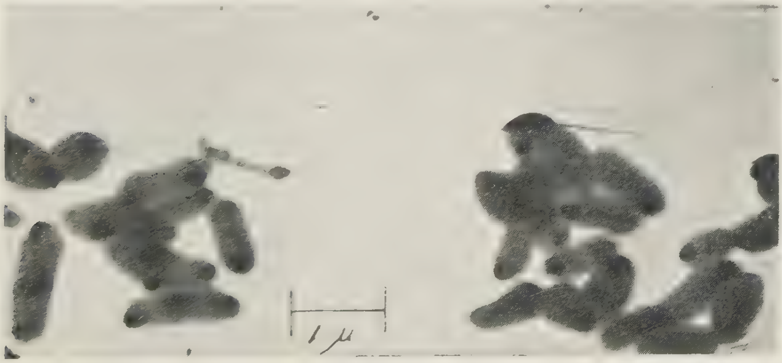

Fig. 1.

*C. diphtheriæ*, XIII, × 20,000. Grown on extract agar, pH 7.2, containing 1% Parke, Davis peptone and 5% normal horse blood, for 48 hours at 37°C and an additional 48 hours at room temperature. Preparations made from a suspension of the bacteria in distilled water.

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[2] Klett, Ad., *Z. f. Hyg.*, 1900, **33**, 137.

[3] Conradi and Troch, *Münch. med. Wochenschr.*, 1912, **59**, 1652.

stained cells of *C. diphtheriae* from blood extract agar. The characteristic polar granules appear as black circles, while the remainder of the cell is light gray. Since, as previously mentioned, electron micrographs reveal differences in densities, this is to be interpreted as indicating that the polar granules are spheres or possibly plates of high density, while the remainder of the cell is of a relatively low density, in the dry state.

Fig. 2 and 3 are of unstained cells grown on potassium tellurite chocolate agar. Inside the cells, and in addition to the polar granules, are seen minute needle-like crystals of high density. If these crystals are actually tellurium metal they should dissolve in the presence of an oxidizing agent such as bromine. To test this point,

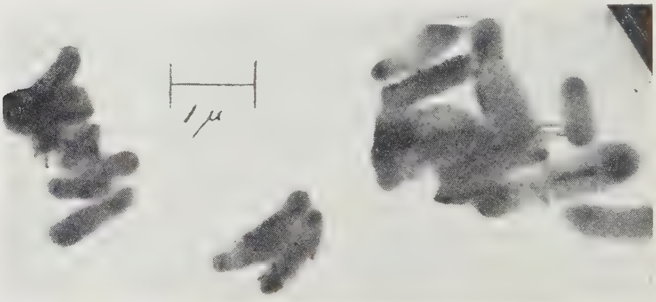

FIG. 2.
*C. diphtheriæ*, XIII, × 18,000. Grown on potassium tellurite chocolate agar (Difco) for 48 hours at 37°C and an additional 96 hours at room temperature. Preparations made from a suspension of the bacteria in distilled water.

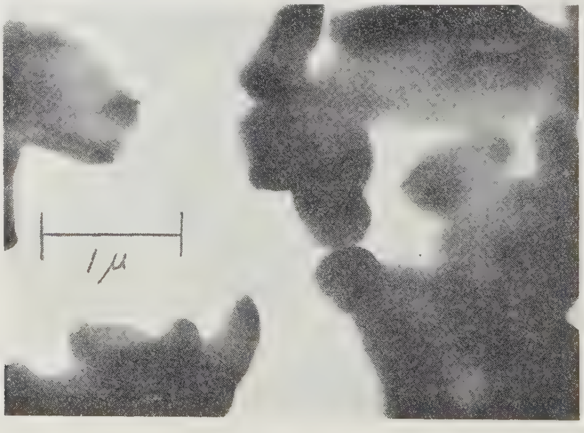

FIG. 3.
*C. diphtheriæ*, XIII, × 30,000. Conditions of growth same as Fig. 2.

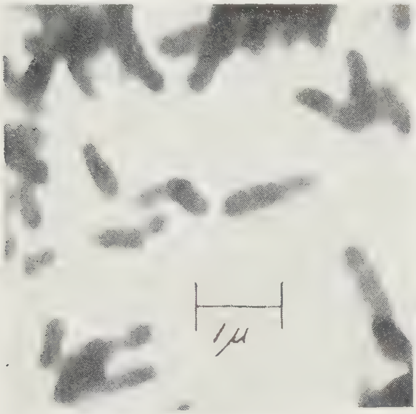

FIG. 4.

*C. diphtheriæ*, XIII, × 18,000. Same as in Figs. 2 and 3, except that the cells, originally containing crystals of tellurium, were treated with bromine water to dissolve the tellurium metal.

a drop of bromine water was added to 1 cc of a suspension of cells from black colonies on potassium tellurite chocolate agar. The black color of the mass of cells immediately disappeared; likewise, the needles were no longer observed in electron micrographs of cells so treated (Fig. 4). Thus it appears highly probable that the black color is due to tellurium metal which exists, at least in part, in the needle-like forms shown in the pictures.

More important than the identification of the crystals is the location of the site of the reduction of the tellurite. In a few exceptional cases (Fig. 3) crystals actually penetrated beyond the cell boundary, possibly during the drying of the preparation. In other cases the crystals distort the cell outline without penetrating. In no cases were crystals observed to lie wholly outside the cells. Since the majority of the crystals are contained wholly within the cells, it is to be inferred that the tellurite or tellurous ion is able to diffuse through the cell wall and is there reduced to tellurium metal which is precipitated inside the cells.

Similar crystals have been observed within cells of *C. xerosis* grown on tellurite chocolate agar.

*Summary.* (1) Typical polar granules appear as dense spherical masses, or possibly plates, in electron micrographs of unstained preparations of *C. diphtheriae* grown on blood infusion agar. (2) In addition to polar granules, needle-like crystals appear in electron micrographs of unstained preparations of *C. diphtheriae* cells grown on potassium tellurite chocolate agar. (3) The needle-like crystals, as well as the black color, of cell masses of *C. diphtheriae* grown on potassium tellurite chocolate agar disappear upon the addition of small amounts of bromine water. It is inferred, therefore, that the black color is due to the tellurium metal which occurs in the form of needles. (4) It is to be further inferred that the tellurite ion is able to diffuse through the cell wall and is reduced with the precipitation of tellurium metal within the cell boundaries. (5) With the aid of the electron microscope it is now possible to obtain pictorial records of the location of sites of certain chemical reactions incident to the metabolism of the bacterial cell.

*Acknowledgments.* We wish to thank Dr. V. K. Zworykin, Associate Director of the Research Laboratories of the RCA Manufacturing Company, for his kind advice and support, and Dr. L. Marton for assistance in obtaining the pictures.

## 11964

## Inactivation of Estrone by Liver After Exclusion of Reticuloendothelial System.*

BERNHARD ZONDEK AND JOACHIM SKLOW.

*From the Hormone Research Laboratory of the Hebrew University, Jerusalem.*

It has previously been established[1] that estrone injected in rats disappears rapidly from the organism without being excreted in the urine and feces. To recover the estrone injected the animals were minced, and from the resulting pap the hormone was extracted by organic solvents. Three hours after the injection less than 5% of the estrone could be extracted, after 20 hours, only 0.2%. Hydrolysis permitted the extraction of as much as 20%. No transformation of the estrone into the less active estriol takes place, the hormone being inactivated in the organism. As has been demonstrated by experiments *in vitro,* this inactivation takes place mainly in the liver, and to a small extent in the spleen. Other organs have no inactivating effect. From the following it was concluded that the inactivation in the liver is a fermentative process (estrinase):

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* Aided by a grant from the Rockefeller Foundation.

[1] Zondek, B., *Lancet,* 1934, **227**, 356; *Skand. Arch. Physiol.,* 1934, **70**, 133; *Hormone des Ovariums und des Hypophysenvorderlappens,* 2nd edition, Springer, Vienna, 1935, p. 124.

(a) The inactivating property of the liver is destroyed at a temperature of over 70°C. (b) A cell-free liver extract still retains the inactivating properties of the liver. (c) The estrone inactivated by liver-pap cannot be reactivated by hydrolysis. That estrogenic hormone is being inactivated by the liver has in the meantime been confirmed by several authors.[2]

The fact that estrone is inactivated by the liver and partly by the spleen, and not by other organs, led to the possibility that the reticuloendothelial system is responsible for this inactivation. We have tried, therefore, to eliminate the function of the reticuloendothelial cells by blocking them. Partial or complete exclusion of this system is possible by means of different substances; coloring agents like methylene-blue or trypan blue, cholesterol, and best by means of heavy metals (copper, iron, gold, etc.). A criterion of the completeness of the "blockade" is the decrease of storing capacity[3] of the reticuloendothelial system. Moreover, it is possible to ascertain the effect of this blocking histochemically.

According to Jansco,[3] the electrocolloidal copper solution (Heyden) given intravenously blocks the reticuloendothelial cells, and in appropriate dosage is able to destroy the whole Kupffer cell system of the liver as well as the reticuloendothelial cells of the spleen and bone-marrow. 0.025 cc of colloidal copper solution (equal to 0.015 mg of copper) are sufficient to eliminate the storing capacity of the Kupffer cells in the mouse. This effect, which begins 10 minutes after injection, reaches its peak in 6 hours. On the basis of these results, we have employed the electrocolloidal copper solution (Heyden) containing 0 06% of copper and a protective colloid. We found that 0.4 cc (0.24 mg of copper) of this solution, injected intracardially in rats weighing 50 g produced death in 48 hours in most cases. This dosage was chosen for our experiment.

*Experiments.* Three infantile rats, weighing 50 g each, received 0.4 cc of copper solution intracardially. Half an hour later the rats received 0.25 mg of estrone each in oily solution, subcutaneously. Control animals not treated with copper, received the same quantity of estrone. Four hours after the administration of the estrone, the animals were sacrificed and reduced to a pap. This, covered with 5 times its amount of acetone, was left for several days in room temperature. The acetone was replaced several times. The acetone removed was evaporized and the sediment (A) was used later. The dry powder left after the removal of acetone was extracted for 24 hours with alcohol in a Soxleth. The alcohol was evaporized, and the remainder (B) added to A, cleaned with alcohol, and the alcohol soluble part dissolved in oil. The experiments revealed that in each of the 3 blocked rats, only 0.005 mg of estrone was to be regained, which means that 98% of the estrone administered was inactivated. It was possible to extract the same amount from the non-blocked control rats. It therefore follows that the exclusion of the reticuloendothelial cells does not affect the inactivation of the estrone in the organism.

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[2] Dingemanse, E., and Laqueur, E., *Am. J. Obst. and Gynec.*, 1937, **33**, 1000; Israel, Meranze, and Johnston, A., *J. Med. Sci.*, 1937, **194**, 835; Golden, J. B., and Sevringhaus, E. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **39**, 361; Talbot, N. B., *Endocrinology*, 1939, **25**, 601; Heller, C. G., *Endocrinology*, 1940, **26**, 619.

[3] Jansco, N., *Klin. Wschr.*, 1931, **12**, 537.

In a second series the duration of experiment was shortened because the exclusion of the reticuloendothelial cells by electrocolloidal copper solution reaches its peak 6 hours after injection, subsequently decreasing.[4] Three rats weighing 50 g received intracardially 0.4 cc (0.24 mg Cu) of colloidal copper solution, and 4 hours later 0.25 mg estrone, subcutaneously in oily solution. Two hours later, *i. e.*, at the peak of "blockade" the animals were sacrificed, and treated in the manner described above. Two control animals received only estrone. These experiments have also led to clearcut results: An average quantity of 0.025 mg of estrone was extracted from each animal in the control as well as the blocked series, *i. e.*, only 10% of the introduced estrone was detected, 90% having been inactivated.

These experiments show that the liver as well as other organs is able to inactivate estrone after exclusion of the reticuloendothelial cells. This fact, together with the above mentioned experiments *in vitro*, shows that the liver cell itself must contain the inactivating factor (ferment) and that the reticuloendothelial cells have no part in the inactivation.

*Summary.* The influence of the reticuloendothelial system on estrone inactivation in the organism has been investigated. It is concluded that the reticuloendothelial cells have no part in this process and that the liver cell in itself is responsible for this inactivation, *i. e.*, contains the inactivating factor (enzyme).

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[4] Letterer, E., *Klin. Wschr.*, 1933, **15**, 597.

11965

## Oxygen Consumption of Brain Cortex in Thyrotoxic Guinea Pigs.

MORRIS A. SPIRTES. (Introduced by M. B. Bender.)
*From the Medical Division Laboratories, Montefiore Hospital for Chronic Diseases, New York City.*

Cohen and Gerard[1] and Macleod and Reiss[2] reported increased oxygen consumption of thyrotoxic brain, the former using whole minced brain of animals fed dried thyroid gland, the latter brain cortex slices of animals fed thyrotropic hormone.

Studies have been made on non-pregnant, white, female guinea pigs, weighing 250-350 g, which, during a period of one week, were daily fed 2 grains of finely ground dried thyroid mixed with cut lettuce, in addition to their regular diet. At the end of this week they had lost one-quarter to one-third of their original weight. They were then killed by a blow on the neck. Slices of brain, liver and kidney were immersed in Ringer solution to which, in the case of the brain slices, 2 cc of 10% glucose and 5 cc $PO_4$ buffer pH 7.4 per 100 cc of solution were added; bicarbonate was omitted. Oxygen consumption was measured by the original Warburg[3] method.

TABLE I.
Average $Q_{O_2}$ of Normal and Thyrotoxic Guinea Pig Tissues.

| Brain | | Liver | | Kidney | |
|---|---|---|---|---|---|
| Normal | Thyrotoxic | Normal | Thyrotoxic | Normal | Thyrotoxic |
| 12.7 | 12.0 | 5.6 | 8.4 | 16.4 | 19.1 |
| 13.5 | 11.8 | 5.8 | 8.6 | 15.2 | 17.7 |
| 13.3 | 11.8 | 7.1 | 9.0 | 14.7 | 18.3 |
| 11.2 | 12.9 | 6.9 | 8.2 | 14.4 | 21.2 |
| 10.5 | 11.9 | 6.9 | 10.1 | 14.0 | 19.8 |
| 10.9 | 12.8 | 4.9 | 8.5 | 14.5 | 21.6 |
| 11.8 | 12.6 | 4.4 | 8.7 | 16.4 | 17.7 |
| 12.3 | 9.6 | 4.3 | 9.4 | 15.4 | 18.0 |
| 11.8 | 13.6 | 6.4 | 9.1 | 16.4 | 18.9 |
| 11.7 | 12.4 | 6.5 | 7.5 | 14.9 | 18.9 |
| 12.5 | 12.3 | 5.5 | 7.3 | 13.8 | 20.8 |
| 11.2 | 12.2 | | 11.4 | 12.9 | 19.3 |
| 11.8 | 11.9 | | 8.8 | | 18.3 |
| | 12.0 | | | | 18.0 |
| | 12.9 | | | | 18.3 |
| | | | | | 19.0 |
| 11.9 | 12.2 | 5.8 | 8.8 | 14.9 | 19.1 |

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[1] Cohen, R. A., and Gerard, R. W., *J. Cell. and Comp. Physiol.*, 1937, **10**, 223.

[2] Macleod, L. D., and Reiss, M., *Biochem. J.*, 1940, **34**, 820.

[3] Warburg, Otto, and Minami, Seigo, *Biochem. Z.*, 1923, **142**, 317, 334.

As seen in Table I, brain-cortex slices of thyroid-fed animals showed no increased oxygen consumption, whereas liver and kidney tissues showed a definite rise.

Fairly large differences in the $Qo_2$ values for one organ in different animals or in the same animal can be found frequently. This error is inherent in the method used and is true especially of organs with low $Qo_2$, such as liver. The values for kidney and brain range about 10-15% on either side of the average, whereas for liver the values range as much as 25% to either side of the average value. It can be seen clearly from the table, however, that the normal and thyrotoxic figures for liver and kidney have entirely different ranges, whereas the range is the same for normal and abnormal brain slices. The average thyrotoxic liver $Qo_2$ is 52% greater than the normal average, that of kidney is 28% higher than the normal average. The average normal and thyrotoxic brain $Qo_2$ are, however, the same.

The foregoing data are at variance with the results obtained by Cohen and Gerard, and Macleod and Reiss. However, all of their results were obtained with rats, whereas the experiments reported here were with guinea pigs. Cohen and Gerard worked with minced, whole brain, and waited 2 hours after the mincing before taking readings for oxygen consumption. In addition, they used a glucose-free Ringer solution. Macleod and Reiss found a normal brain cortex $Qo_2$ of 7.3, which is far below the accepted normal of 10 to 11.[4, 5] This makes the validity of their results questionable. Even their values for hyperthyroid brain do not exceed the accepted normal.

To ascertain further whether brain cortex is exceptional in its response to thyroid hormone, experiments are in progress with thyroidectomized guinea pigs.

*Conclusion.* The *in vitro* oxygen consumption of brain-cortex slices from thyrotoxic guinea pigs is not above normal.

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[4] Unpublished figures of the author for rat brain cortex.

[5] Warburg, Otto, *Biochem. Z.*, 1924, **152**, 309.

## 11966 P

# Entrance of Radioactive Phosphorus into Sphingomyelin of Various Tissues of Cat.

F. E. Hunter. (Introduced by W. R. Bloor.)

*From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, The University of Rochester, Rochester, New York.*

Measurement of the rate of uptake of radioactive phosphorus by the total phospholipid fraction of various organs has been carried out by many investigators.[1] A few reports have appeared in which lecithin and cephalin have been separated for the determination of radioactivity in the individual phospholipids.[2] This report is concerned with the measurement of the rate of entrance of radioactive phosphorus into the phospholipid sphingomyelin. Such data should give an indication of the part which sphingomyelin plays in total phospholipid phosphorus metabolism and of what its rôle in the body may be. Since this work was undertaken, one report of a similar measurement has appeared. Chargaff, *et al.*,[2] measured the activity in the sphingomyelin fraction of the brain of rats and found the phosphorus turnover for sphingomyelin to be about the same as for brain cephalin.

In order to be able to obtain sufficient sphingomyelin, the cat was chosen for the experimental animal. Seven young female animals were used. They were killed at 1, 2, 4, 6, and 8 days after receiving by stomach tube 5 to 10 mg of radioactive phosphorus in the form of a solution of $Na_2HPO_4$.

The fresh tissues (brain, kidney, heart, spleen, lung, intestinal mucosa, liver, and skeletal muscle) were ground with sand and extracted with alcohol, twice with 3:1 alcohol-ether, and twice with 1:1 chloroform-methanol. The extracts were combined and the solvent removed under diminished pressure. The lipids were dissolved in petroleum ether containing a little chloroform and any residue centrifuged out. Sphingomyelin, in itself rather insoluble in petroleum ether, is carried into solution at room temperature by the large amount of other lipids present.[3] It was isolated by a micromodification of the method published by Thannhauser, *et al.*[4] This modification, worked out in this laboratory for these determinations, is almost identical with that published recently by Erickson, Avrin, Teague, and Williams[5] and need not be further described. The phosphorus analyses were performed by the method of Kuttner and Lichtenstein.[6] The radioactivity measurements were made according to the methods in general use in this laboratory.[7]

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[1] Artom, C., Sarzana, G., Perrier, C., Santangelo, M., and Segre, E., *Arch. internat. physiol.*, 1937, **45**, 32; Artom, C., Sarzana, G., and Segre, E., *Arch. internat. physiol.*, 1938, **47**, 245; Perlman, I., Ruben, S., and Chaikoff, I. L., *J. Biol. Chem.*, 1937, **122**, 169; Chargaff, E., *J. Biol. Chem.*, 1939, **128**, 587.

[2] Chargaff, E., *J. Biol. Chem.*, 1939, **128**, 587; Chargaff, E., Olson, K. B., and Partington, P. F., *J. Biol. Chem.*, 1940, **134**, 505.

[3] Haven, F. L., personal communication; Taylor, J. D., personal communication.

The radioactive phosphorus content of the sphingomyelin reaches a maximum within 8 days in all tissues except the brain and the skeletal muscle. In the latter 2 tissues the uptake of radioactive phosphorus by sphingomyelin is very slow and continues for more than 8 days. In the liver, lung, and intestinal mucosa the maximum is reached at 2 days after feeding. The maximum appears to come at about the same time, perhaps a little later, in the kidney, spleen, and heart. Expressed as percent of administered dose per mg of sphingomyelin phosphorus the maxima (averages from the values on 2 animals in each case) are as follows: liver 0.021, lung 0.017, intestinal mucosa 0.016, kidney 0.012, spleen 0.012, and cardiac muscle 0.0065. At the end of 8 days the value for skeletal muscle is about 0.0012 and for brain 0.0008.

Preliminary data on the liver indicate that the activity of the total phospholipid fraction reaches a maximum during the first day. This maximum is 0.17% of the dose of radioactive phosphorus fed and is about 8 times as great as that for sphingomyelin.

*Conclusions.* Radioactive phosphorus is incorporated into sphingomyelin to the greatest extent in the liver, the lung, and the intestinal mucosa. With the exception of the lung the tissues distribute themselves in approximately the same order for sphingomyelin activity as for total phospholipid activity. The lung is relatively higher in the case of sphingomyelin. Radioactivity in sphingomyelin reaches a maximum at about two days in most tissues. In the case of liver the total phospholipid fraction appears to be many times more active than sphingomyelin, indicating that this phospholipid, though possessing a certain definite activity, plays only a small part in phospholipid phosphorus metabolism in this organ.

The author is indebted to Miss Sylvia Levy for one-half of the radioactivity measurements and to Dr. William F. Bale and Dr. S. N. Van Voorhis for preparation of radioactive phosphorus.

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[4] Thannhauser, S. J., and Setz, P., *J. Biol. Chem.*, 1936, **116**, 533; Thannhauser, S. J., Benotti, J., and Reinstein, H., *J. Biol. Chem.*, 1939, **129**, 709.

[5] Erickson, B. N., Avrin, I., Teague, D. M., and Williams, H. H., *J. Biol. Chem.*, 1940, **135**, 671.

[6] Kuttner, T., and Lichtenstein, L., *J. Biol. Chem.*, 1930, **86**, 671.

[7] Bale, W., Haven, F., and LeFevre, M., *Rev. Scient. Instruments*, 1939, **10**, 193.

### 11967

# Effect of Testosterone Propionate on Anal Fin Transformation of Female Viviparous Teleost, Xiphophorus Helleri Heckel.

FRANCIS MARSH BALDWIN AND HOWARD SIDNEY GOLDIN.
*From the Physiological Laboratories, University of Southern California, Los Angeles.*

While working on another problem, Essenberg[1] observed and recorded 2 cases of natural complete sex-reversal in this species, from female to male. He reported that the anal fin of the normal female consists of 10 pairs of rays, all of which are approximately of the same length and diameter. During the period of natural sex-reversal, he also observed a medial thickening of the third pair of rays, as well as elongation of the third, fourth, and fifth pairs of rays, to approximately twice the length of the original fin. The first, second, and sixth to tenth pairs of rays remained constant in length. The apex of the third pair of rays together with the fourth and fifth pairs formed hooks and counterhooks for anchorage to the female genital pore during copulation.

In connection with another study of experimentally induced sex-reversal in the female of the same species, Baldwin and Goldin[2] treated 91 virgin females with testosterone propionate* over a period of approximately 19 weeks. Controls treated with pure sesame oil showed no response. Each treated fish received 0.5 mg testosterone propionate in 0.02 cc sesame oil weekly. This treatment causes, among other changes,[2] the transformation of the normal female anal fin into an organ that simulated in structure the male copulatory organ, the gonopod.

*Experimental.* The normal anal fins of the experimental female fish consist of 10 pairs of rays of approximately the same length and diameter. In general, after administration of testosterone propionate, the third pair of rays thickened to several times their normal diameter. The third, fourth, and fifth pairs of rays elongated to approximately twice the original length of the fin. In addition, the tips of the third, fourth, and fifth pairs of rays form knob-like projections which appear to be the anlage of the copulatory hooks of later development. Subsequently, these knob-like projections appear to have grown slightly to form bulges on the dorsal and ventral margins of their respective rays. The knobs of the ends of the third and fifth pairs of rays develop into primitive copulatory hooks which soon become fully developed. The knob-like projections of the fourth pair of rays appear to have undergone an early development to form accessory copulatory hooks, or counterhooks, to supplement those of the third and fifth pairs of rays. These accessory hooks which began their development just previously, soon appear as fully formed accessory copulatory hooks. Symmetrical teeth-like projections are noted on the third, fourth, and fifth pairs of rays. Thus, the experimentally induced changes in the normal female anal fin simulate the structural configuration of the normal male anal fin, referred to as the gonopod.

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[1] Essenberg, J. M., *Biol. Bull.*, 1926, **51**, 98.

[2] Baldwin, F. M., and Goldin, H. S., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 813.

* By the courtesies of Dr. G. Stragnell of the Schering Corporation, and of the Ciba Pharmaceutical Products, Inc.

*Conclusions.* The transformation of the normal female anal fin into a male-like gonopod is induced by prolonged administration of testosterone propionate. Such treatments influence this secondary sex characteristic in all cases, from female to male, over an interval of approximately 5 months. These experimentally induced transformations of the normal female anal fin into that of a male-like gonopod were observed in all of 91 specimens so treated.

## 11968 P

## Secretion of Orally Administered Radio-Iron in the Milk of Cows.

L. A. ERF.* (Introduced by J. H. Lawrence.)

*From the Crocker Radiation Laboratory, University of California, Berkeley.*

The purpose of this experiment was to determine the percentage of ingested iron taken up by the whole blood and that secreted into the whole milk of cows.

*Methods.* Radio-iron ($_{26}Fe^{59}$) produced in the Berkeley cyclotron was converted into an iron chloride solution,[1] each cc of which contained 4 mg of $FeCl_3$ and emitted, on the day of administration, 1820 counts/sec. of gamma radiation. (Only gamma radiation was measured.)

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* Wm. R. Kenan, Jr., Fellow.

[1] Austoni, M. E., Rabinovitch, A., and Greenberg, D. M., *J. Biol. Chem.*, 1940, **134**, 17.

Two cows were used, one a grade Holstein, No. 238, weighing 800 lb and in the 8th month of the 2nd lactation period, the other a grade Jersey, No. 684, weighing 650 lb and in the 6th month of the 1st lactation period. The former had 12 g of hemoglobin per 100 cc of blood before and after the experiment, the latter 13.1 g. Both cows had received, for months, the same diet. No. 684 received twice daily for 12 days immediately preceding the experiment, 8 g of ferrous sulphate.

*Experiment.* The cows were milked dry just before the oral administration (by drenching) to each of 115 cc of the labeled iron chloride solution. Six hours later both were milked and bled. They were milked twice daily thereafter and the total milkings saved separately for 3 days. One hundred cc of whole blood were removed from each on the 6th, 30th, 48th and 78th hours. The 8 samples of whole milk and the 8 of whole blood were ashed at 850°C and together with aliquot samples of the solution administered were assayed by means of a modified Geiger-Muller counter.

*Results.* Table I indicates the results.

TABLE I.

| Cow | Hrs after oral admin. of $_{26}Fe^{59}$ | Cc of specimens obtained | Gamma radiation emitted by ashed specimens (counts/sec) | Recovery of $_{26}Fe^{59}$ admin. in % per L whole blood or whole milk | Avg % admin. radio-iron per L of milk secreted during 78 hr |
|---|---|---|---|---|---|
| No. 238 | | | | | |
| A. Blood | 6 | 100 | 20 | .095 | |
| | 30 | " | 23 | .110 | |
| | 48 | " | 28 | .133 | |
| | 78 | " | 17 | .081 | |
| B. Milk | 1- 6 | 1220 | 1680 | .65 | |
| | 6-30 | 3570 | 851 | .11 | |
| | 30-48 | 4560 | 466 | .05 | |
| | 48-78 | 4000 | 420 | .05 | |
| | | 13350 | | | .121 |
| No. 684 | | | | | |
| A. Blood | 6 | 100 | 16 | .076 | |
| | 30 | " | 30 | .143 | |
| | 48 | " | 28 | .128 | |
| | 78 | " | 32 | .152 | |
| B. Milk | 1- 6 | 2300 | 990 | .22 | |
| | 6-30 | 4930 | 2502 | .24 | |
| | 30-48 | 5900 | 442 | .03 | |
| | 48-78 | 5680 | 1170 | .10 | |
| | | 18810 | | | .128 |

*Whole Blood.* The levels of radio-iron in the blood of each were quite similar despite the fact that the iron reservoirs of No. 684 supposedly were adequately saturated. The levels were similar to those found in dogs[2] but lower than those of rats.[1]

*Whole Milk.* The configuration of the curve illustrating the amount of radio-iron secreted in milk of No. 238 is similar to those which show the amount of radio-strontium secreted in milk.[3] The curve of No. 684 is flatter. No. 238 produced 13,350 cc of milk and secreted an average of .121% of radio-iron per liter of milk, or a total of 1.5% of the amount administered, during the 78-hour period; while No. 684 produced 18,810 cc, and secreted .128% per liter, or a total of 2.5% of labeled iron.

*Discussion. Blood.* The previous administration of non-radioactive iron apparently did not lower the amount of radioactive iron absorbed, probably because the iron reservoirs of both cows were saturated. However, there were differences in breed, period of lactation and probably in rates of bodily metabolism and rates of utilization of iron.[4] The ratios of radio-iron in the plasma and red cells should have been made for undoubtedly such results would have explained the more rapid rate of secretion of radio-iron in the milk during the first few hours after the administration of ${}_{26}Fe^{59}$. It has been demonstrated that absorbed radio-iron is present first in the plasma and later fixed in the hemoglobin of the red cells[2, 5] and that the kidney cells excrete radio-iron only when the plasma levels are high.[6]

*Milk.* Similarly it can be assumed that radio-iron is secreted by the lactating cells only when the level of plasma radio-iron, which is available to the cells, is high. The rate of the secretion of radio-iron in the milk was slower and more prolonged in No. 684 than in No. 238. This would make one suspect that the non-radioactive iron previously administered had saturated the body cells and fluids and overtaxed the excretory mechanism, thereby permitting the labeled iron a prolonged exposure to the lactating cells of this cow. The greater total quantity of radio-iron secreted by this cow was probably due to the supposition just mentioned and to the fact that she produced more milk and was in an earlier state of lactation.

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[2] Hahn, P. F., Bale, W. F., Lawrence, E. O., and Whipple, G. H., *J. Exp. Med.*, 1939, **69**, 739.

[3] Erf, L. A., and Pecher, C., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 762.

[4] Hahn, P. F., Ross, J. F., Bale, W. F., and Whipple, G. H., *J. Exp. Med.*, 1940, **71**, 731.

[5] Miller, L. L., and Hahn, P. F., *J. Biol. Chem.*, 1940, **134**, 585.

[6] Hahn, P. F., Bale, W. F., Hettig, R. A., Kamen, M. D., and Whipple, G. H., *J. Exp. Med.*, 1939, **70**, 443.

If the average cow producing 4 liters of milk a day can secrete in the milk 0.5% of a 10 g oral dose of iron per 24 hours, 12.5 mg of iron would be present in each liter—an amount more than adequate for a growing child who consumed a liter of milk a day.

*Conclusions.* In the 2 cows studied, 1.5% and 2.5% of the radioiron administered orally was secreted respectively in the milk during a 78-hour period.

I am deeply indebted to Dr. M. D. Kamen for preparing the radioactive iron solution used and for counting the samples; to Dr. O. W. Schalm, Department of Veterinary Science, for the use of the cows and for the procurement of the blood and milk used; and to Dr. L. H. Duschak, Department of Mining Engineering, for use of high temperature ovens.

### 11969 P

## NaCl and Peritoneal Absorption and Renal Excretion of Glucose in Normal and Diabetic Rats.*

George Sayers and James M. Orten.

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Sodium chloride appears to influence the glucose tolerance of the diabetic rat[1] and to exert a favorable effect upon certain human diabetics.[2] Its mode of action is interesting from both the practical and the more theoretical aspects of carbohydrate metabolism. It may aid the body in storing[3] and in utilizing more glucose or it may lower the absorption and retention[4] of sugar.

The present study has been made to determine the effect of sodium

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* The data in this paper were taken from a dissertation presented by George Sayers in partial fulfillment of the requirements for the degree of Master of Science in Wayne University, 1941.

Aided by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[1] Orten, J. M., and Devlin, H. B., *J. Biol. Chem.*, 1940, **136**, 461.

[2] McQuarrie, I., Thompson, W. H., and Anderson, J. A., *J. Nutrition*, 1936, **11**, 77.

[3] Crabtree, D. G., and Longwell, B. B., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 705.

[4] McDanell, L., and Underhill, F. P., *J. Biol. Chem.*, 1917, **29**, 273.

chloride on the peritoneal absorption and urinary loss of glucose in rats administered glucose intraperitoneally under standardized conditions. Normal and hereditary diabetic rats of the "Yale" strain, classified by tolerance studies, and partially pancreatectomized rats were used. After a 16- to 18-hour fast, either glucose alone or a glucose solution containing sodium chloride (see Table I) was injected intraperitoneally; the rats were placed in special metabolism cages and at the end of a 5-hour period the blood sugar was determined by the micro method of Hagedorn and Jensen[5] upon blood obtained from a tail vein. The animals were then lightly anesthetized with nembutal and all residual urine in the bladder was removed by catheterization. In sacrifice experiments, the peritoneal fluid was carefully withdrawn and measured. Hanes' modification[6] of the Hagedorn and Jensen method was used to determine urinary and peritoneal fluid sugar.

The data obtained on individual animals and the group averages are given in Table I. The apparent increase in glucose tolerance brought about by sodium chloride confirms the previous report from this

TABLE I.
Effect of Sodium Chloride on Absorption and Excretion of Intraperitoneally Injected Glucose* During 5-hour Period.

| | Normal | | Hereditary diabetics | | Pancreatectomized | |
|---|---|---|---|---|---|---|
| | Glucose alone | Glucose +NaCl | Glucose alone | Glucose +NaCl | Glucose alone | Glucose +NaCl |
| Terminal blood sugar, avg (mg%) | 134 | 121 | 226 | 122 | 193 | 135 |
| Glucose absorbed (%) | 80 | 86 | 82 | 84 | 88 | 84 |
| | 86 | 75 | 88 | 72 | 75 | 83 |
| | 91 | 94 | 82 | | | 81 |
| | 80 | | | | | |
| Avg | 84 | 85 | 84 | 78 | 82 | 83 |
| Urinary glucose (mg) | 3 | 53 | 0 | 81 | 0 | 217 |
| | 9 | 291 | 9 | 49 | 25 | 161 |
| | 5 | 62 | 4 | 81 | 7 | 179 |
| | 2 | 230 | 8 | 49 | 3 | 207 |
| | 3 | 172 | 15 | 95 | 18 | 279 |
| | | | 3 | 39 | 13 | 120 |
| | | | 7 | | | |
| Avg per 100 g body wt. | 1.2 | 52 | 2.4 | 23 | 4.0 | 75 |

*4 cc/100 g body wt. of either an 8.75% glucose alone or an 8.75% glucose + 1.58% NaCl solution.

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[5] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46.

[6] Hanes, C. S., *Biochem. J.*, 1929, **23**, 99.

laboratory.[1] While sodium chloride had no significant effect upon glucose absorption from the peritoneal cavity, it produced a marked glycosuria in all rats. This was especially striking in the pancreatectomized animals.

In preliminary studies, the data of which are not included in the present paper, a similar glycosuria and a normal glucose tolerance resulted when the glucose was given in an 0.85% sodium chloride solution to the normal and diabetic rats. The effects were somewhat less pronounced, however, than were those obtained with the stronger concentration of sodium chloride (Table I).

Some striking differences between the animals given glucose alone and those given added sodium chloride were observed. Bleeding from the tail was extremely difficult in the former group and both the water intake and urine output were practically *nil,* as has been reported by Darrow and co-workers[7] for other species similarly treated. The rats given added sodium chloride, on the other hand, bled normally, drank freely, and excreted relatively large amounts of urine. It appears that the extra water intake of the sodium- chloride-treated rats resulted in the diuresis which in turn was accompanied by the observed increased glycosuria. That sodium chloride has more than a glycosuric action, however, is indicated by preliminary studies which show that despite the high urinary loss of glucose there is an increase in the storage of glycogen in the livers of the sodium chloride-treated rats.

*Summary.* Sodium chloride added to an intraperitoneally injected glucose solution has no influence upon the absorption of the glucose but does increase glucose excretion by way of the kidney. This increased glycosuria appears to be a factor involved in producing the normal glucose tolerance observed in hereditary diabetic and partially-pancreatectomized rats administered sodium chloride with the glucose.

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[7] Darrow, D. C., and Yannet, H., *J. Clin. Invest.*, 1935, **14**, 266.

## 11970

# Effect of Soybean Oil Meal on Avian Reproduction.*

H. S. Wilgus, Jr., and F. X. Gassner.

*From the Colorado State College Experiment Station, Fort Collins, Colo.*

In studies of the iodine requirements of poultry a simple type of goitrogenic ration has been used at this Station. This has consisted essentially of ground yellow corn, dried brewers' yeast residuals, soybean oil meal, steamed bone meal, salt and vitamin D oil. This has supported fairly good growth and feathering and has allowed the production of severe goiter when feedstuffs selected for low iodine content were used. The goitrogenic effect has been readily overcome by the addition of iodine to the ration. However, it was found that both production and reproduction on this ration were poor, even when sufficient iodine was present to prevent goiter.

In order to study the iodine requirements of laying birds, it was necessary to correct this difficulty. Experience had shown that the replacement of part of the soybean oil meal with casein in the type of ration just mentioned would permit excellent growth. Accordingly, a number of single-comb White Leghorn pullets, reared on the goitrogenic type of ration, were divided into 3 pens at time of sexual maturity and were fed rations containing different proportions of casein and soybean oil meal. The protein, vitamin, calcium and phosphorus content of these rations were approximately the same. Oat groats were also added at the 15% level in pens 2 and 3. Pertinent data are given in Table I.

It is obvious from the data in Table I that reproductive performance in period A improved as the soybean oil meal was replaced with casein and oat groats. Nevertheless the results were not satisfactory. Hence in period B the amount of soybean oil meal was reduced to 6% in pens 1 and 2. Pen 3 was retained as control on the same level of soybean oil meal as in period A. Pen 2 received an addition of bran since Taylor and Lerner[1] had reported that it aided in hastening sexual maturity. Pen 1 received bran and meat scrap in place of casein.

While bran improved the ration, the beneficial response to bran and meat scrap was marked. No difference was noted in thyroid weight and histology. This indicated that the small amount of

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* This is Paper 115 of Scientific Journal Series, Colorado Experiment Station.

[1] Taylor, Lewis W., and Lerner, I. Michael, *Poultry Sci.*, 1939, **18**, 323.

TABLE I.

| Exp. No. | Pen | Protein supplements | Birds, No. | Egg wt., g | Egg prod., % | Eggs set No. | Fertility % | Hatchability % |
|---|---|---|---|---|---|---|---|---|
| 1 | | Period A—24-31 weeks of age | | | | | | |
| | 1 | 16% soybean oil meal | 21 | 43 | 40 | 280 | 68 | 8 |
| | 2 | 12% ” ” ”, 1% casein | 21 | 43 | 39 | 291 | 80 | 31 |
| | 3 | 6% ” ” ”, 4% ” | 21 | 45 | 37 | 259 | 71 | 54 |
| | | Period B—32-41 weeks of age—6% soybean oil meal in each ration | | | | | | |
| | 1 | 20% bran, 6% meat scrap | 18 | 52 | 59 | 498 | 90 | 67 |
| | 2 | 20% ” 4% casein | 18 | 50 | 49 | 403 | 88 | 51 |
| | 3 | 4% casein | 16 | 52 | 37 | 204 | 84 | 35 |
| | | Period C—42-59 weeks of age—6% soybean oil meal and mixed cereals in each ration | | | | | | |
| | 1 | 5% meat scrap | 15 | 57 | 29* | 355 | 90 | 70 |
| | 2 | 2% casein | 14 | 55 | 27* | 326 | 92 | 63 |
| | 3 | 2% casein | 13 | 56 | 24* | 258 | 81 | 55 |
| 2 | | 24-34 weeks of age—6% soybean oil meal in each ration | | | | | | |
| | 1 | 4% casein | 18 | 44 | 31 | 441 | 65 | 20 |
| | 2 | 4% ” + 2,000 p.p.b. iodine | 7 | 46 | 31 | 140 | 60 | 0 |
| | 3 | 4% ” + 50,000 p.p.b. ” | 18 | 45 | 33 | 365 | 78 | 17 |
| | 4 | Practical ration | 25 | 50 | 41 | 719 | 91 | 72 |

*Slump caused by moving birds.

iodine contained in the bran and meat scrap was not responsible for the beneficial effects.

In period C all pens received a mixed cereal base of ground yellow corn, wheat bran, wheat gray shorts and pulverized oats. Pen 1 continued to receive meat scrap as the chief protein supplement but with dried buttermilk replacing the yeast. Pens 2 and 3 received casein and yeast. While the data show that the mixed cereal base influenced reproduction favorably, meat scrap was still essential for better results. Subsequent studies have confirmed this observation. Furthermore, early work has indicated that dried buttermilk would have little beneficial effect.

Further evidence was obtained in Experiment 2. The birds in pens 1, 2, and 3 were reared on the goitrogenic ration with iodine supplementations as indicated in the table. The reproduction of these birds on this type of ration was very poor, even with the addition of iodine. In contrast to this was the satisfactory performance of control pen 4, which was fed the practical ration. This ration contained 6% of soybean oil meal with the mixed cereal base, meat scrap and dried buttermilk.

In both experiments chicks hatched from eggs of hens on the experimental ration which did not contain any meat scrap were very poor. Few survived the first week, even when fed a practical ration. Chick condition and viability were proportional to hatchability.

It is evident from these preliminary observations either that soybean oil meal exerts a depressing effect on reproduction or that meat scrap, bran, shorts and possibly casein tend to correct a deficiency in the ration. Particularly surprising is the fact that this goitrogenic type of ration with casein will support an excellent rate of growth while it fails to maintain satisfactory production, egg size, fertility and hatchability.

The detrimental effect from soybean oil meal is probably not the same as that noted from cottonseed oil by Morgan and Ringrose.[2]

A number of other workers have demonstrated that soybean oil meal as the sole protein supplement in poultry rations fails to support good hatchability. This was earlier ascribed to a deficiency of riboflavin. Christiansen, Halpin and Hart[3] recently demonstrated that such rations may be deficient in manganese as well as in riboflavin. Nestler, Byerly, Ellis and Titus[4] presented data indicating that poor hatchability with soybean oil meal was not corrected by supplementing the rations with dried whey, a good source of vitamin G. They postulated the existence of a newly discovered factor required for hatchability present in green grass, dried pork liver and to a lesser extent, in meat and fish by-products. The poor hatchability encountered in our work could not readily have been caused by a deficiency of riboflavin or of manganese, since sufficient yeast was used to provide a total of 240 to 260 units per 100 g of ration and since 50 parts per million of manganese was added. Byerly, Titus, Ellis and Nestler[5] have reported that hatchability of eggs from birds on the soybean oil meal ration is increased when these birds get direct sunlight. Christiansen, *et al.*,[3] have noted poorer hatchability on such a ration during the winter months. We have not ascertained as yet whether or not sunlight would have an effect similar to that of meat scrap.

It has been shown by Sharpless, Pearson and Prato,[6] and has been substantiated by this Station,[7] that soybeans have a marked goitrogenic effect. Soybean oil meal has also been shown to be goitrogenic.[7] It is suggested that there may be a relationship between the goitrogenicity of soybean oil meal and the poor reproduction noted in this report This goitrogenic factor could act primarily as a stimulant on the hypophysis. Excess of the thyrotropic hormone thus produced would account for the marked hyperplastic and hypertrophic changes in the thyroid glands of birds on a low iodine ration. It would also account for the depressed reproduction, either through excess thyroxine, or through an excess of gonadotropic hormone, or both. The fact that the thyroid gland is closely interrelated to the ovary in the reproducing female would lend additional support to this assumption.

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[2] Morgan C. L., and Ringrose, R. C., 51st Ann. Rpt., So. Car. Exp. Sta., 1939.

[3] Christiansen, J. B., Halpin, J. G., and Hart, E. B., *Poultry Sci.*, 1940, **19**, 55.

[4] Nestler, R. B., Byerly, T. C., Ellis, N. R., and Titus, Harry W., *Poultry Sci.*, 1936, **15**, 67.

[5] Byerly, T. C., Titus, H. W., Ellis, N. R., and Nestler, R. B., *Poultry Sci.*, 1937, **16**, 322.

[6] Sharpless, George R., Pearsons, Janice, and Prato, Geneva S., *J. Nutr.*, 1939, **17**, 545.

[7] Wilgus, H. S., Jr., Gassner, F. X., Patton, A. R., and Gustavson, R. G., unpublished results.

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### 11971 P

## Experimental Infection of the Rabbit with Duval's Chromogenic *B. leprae* Culture.

WALKER L. LOVING. (Introduced by C. W. Duval.)

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Many attempts have been made to infect the lower animal with fresh human leprosy tissue rich in the bacillus of Hansen and with acid-fast cultures isolated from the lesion of human leprosy. While Sugai,[1] Kedrowski,[2] Duval,[3] *et al.*, claim that leprosy may be induced experimentally, there is no mention of a progressive type of infection having occurred.

Since doubt exists as to the causal significance of Duval's chromogenic acid-fast culture of leprosy, the author has attempted to clear up the matter through a comparative study of the lesions produced in the rabbit with Duval's culture and certain well known saprophytic acid-fast bacilli and to determine whether the experimental infection is progressive and becomes generalized.

Saline suspensions of Duval's chromogenic bacillus of human leprosy and cultures of *B. phlei* and *B. smegmatis* were used for the injection of the animals. Fifty normal full grown rabbits were employed. The animals were inoculated either subcutaneously, intravenously or intraperitoneally with 2 cc of a heavy suspension of the various cultures. Each animal received 3 injections in all at weekly intervals over a period of 3 weeks. The animals were sacrificed at varying intervals and autopsies were performed for cultural and tissue studies. Sections of the organs from the autopsied animals were properly prepared for microscopic studies.

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[1] Sugai, T., *Lepra,* 1909, **8**, 203.

[2] Kedrowski, W. J., *Ztschr. f. Hyg.*, 1901, **37**, 52.

[3] Duval, C. W., *J. Exp. Med.*, 1910, **13**, 374.

Special cultural methods were employed in the recovery of the acid-fast bacilli from the infected animals, especially where recovery of the bacillus was attempted months after the infection had been induced.

It is significant that the cultivation of Duval's acid-fast bacillus from the experimental lesion was difficult in direct proportion to the microbe's sojourn in the tissues of the host. In the instance where lesions were produced with the acid-fast saprophytes, cultures of these readily grew out in 24 hours upon the ordinary laboratory media.

Early in the infection the recovered culture was obtained from the lesions in 48 hours at incubation of 37°C, while 20 days were required to obtain growth on special culture media where the infection had lasted for 6 months.

Duval and Harris report that saprophytic acid-fast microbes produce lesions in the rabbit only where repeated massive doses are injected. No lesions were noted or cultures recovered in any of my *B. phlei* or *B. smegmatis* animals that were sacrificed 3 months after the last injection.

While the gross lesions in the various rabbits inoculated with Duval's culture were not typical of human leprosy, the microscopic tissue changes were identical. Constituting the lesions were aggregations of lymphoid, epithelioid and the so-called "lepra" or "foamy" cells, whose nuclei were crowded to the periphery by the intracytoplasmic colonization of acid-fast bacilli. Free extracellular acid-fast bacilli were also observed. Extensive fibrosis was noted in many of the affected tissues.

*Summary.* Duval's chromogenic acid-fast culture recovered from the tissues of human leprosy produced lesions in the rabbit that microscopically are identical to those of the human disease. The infection is progressive over a period of 6 to 8 months. It is significant that the causal microörganism steadily increases in number throughout this period.

The chromogenic acid-fast bacillus of Duval was recoverable from the experimental lesions as late as 8 months after the last inoculation. *In vitro* cultivation was more difficult in direct ratio to its residence in the host animal.

In the control animals *(B. phlei* and *B. smegmatis)* no gross or microscopic lesions were observed one month after the last injection. No culture was obtained or acid-fast bacilli observed in the tissues of the control animals which were sacrificed 3 months after the last injection.

The results of the experiments herein reported show that Duval's chromogenic acid-fast bacillus induces a chronic form of infection in the rabbit with characteristic tissue changes deep in the parenchyme of the various organs.

## 11972

## Further Studies in the Elimination of Certain Dyes by Gastric Mucosa.

RICHARD L. VARCO AND M. B. VISSCHER.

*From the Department of Physiology, University of Minnesota, Minneapolis, Minn.*

Studies on the elimination of dyes by the gastric mucosa have been made by several investigators with general agreement in results except for the report of Morrison, *et al.*[1] A comprehensive list of dyes was investigated, and their secretion into the stomach noted by Dawson and Ivy,[2] Kobayashi,[3] and Ingraham and Visscher.[4] The last named investigators confirmed the conclusion of Kobayashi that only basic dyes, *i. e.*, those having the chromogen in the electro-positive ion, are secreted by the gastric glands. In addition, they clarified the anomalous intragastric appearance of certain dyes, ordinarily classed as acid, following their intravenous injection (noted by Dawson and Ivy). This previously inexplicable phenomenon has been shown to be due to the amphoteric characteristics of certain dyes, and to alterations of charge on the chromogen upon reversible reduction. A review of the data of Dawson and Ivy, Kobayashi, and Ingraham and Visscher, with these facts in mind, indicates general agreement and leads to the conclusion that only those dyes in which the chromogen can be electro-positive are secreted by the stomach. In a more recent study Morrison, Reeves, Gardner[1] have reinvestigated the excretion of dyes by the gastric mucosa in Pavlov pouch dogs. Congo red, an acid colloidal dye, as well as 8 other acid dyes, were found by these workers to be so excreted. The disagreement of these results with those of other workers has prompted the reinvestigation herewith reported.

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[1] Morrison, Samuel, Reeves, David L., and Gardner, Raymond, E., *Am. J. Dig. Dis. and Nut.*, 1936, **3**, 551.

[2] Dawson, A. B., and Ivy, A. C., *Am. J. Physiol.*, 1925, **73**, 304.

[3] Kobayashi, K., *Acta scholae Med. Univ. Imp. Kioto*, 1926, **8**, 465.

[4] Ingraham, Raymond C., and Visscher, Maurice B., *J. Gen. Physiol.*, 1935, **18**, 695.

Medium size, healthy, mongrel dogs, possessing a functioning Heidenhain or Pavlov pouch which had been constructed a minimum of three months prior to these experiments, were employed. The animals received a varied diet including an extra quantity of sodium chloride, and maintained a good nutritional status. All dogs were trained to remain standing for the duration of the particular experiment. Gastric secretion was stimulated by a subcutaneous injection of histamine diphosphate. (0.2 mg/kg) given in a divided dose or by an intramuscular injection of 10 mg of the histamine base in a beeswax mixture.[5] The secretion was collected in a test tube suspended from the animal so as to allow for free flow from the pouch via a mushroom type catheter. The fluid thus collected was studied for free and total acidity by titration with 0.1 N NaOH using Töpfers reagent and phenolphthalein. When the value of the former approximated 140 m equiv. per l, the dye to be tested was injected intravenously in aqueous solution. In every instance the quantity and dilution of the dye given was that used by Morrison and his coworkers. Successive samples were then collected for the following 1 to 2 hours, the amount measured, an aliquot taken for titration, and another specimen alkalinized with 0.1 N NaOH and any color change noted, a final sample was oxidized with hydrogen peroxide and observed for color change. The following dyes were investigated: Orange G, Congo Red, Trypan Red, Eosin, Mercurochrome, Erythrosine, Phloxine, Rose Bengal, Lithium Carmine. All dyes were tested by injection into 2 different animals, excepting Congo Red, which was used 7 times.

The detailed observations are shown in Table I. Upon no occasion did any animal eliminate any acid dye in this series. In order to verify the activity and dye secretory capacity of these pouches, chrysoidin, a basic dye, was injected intravenously, under similar test conditions, and in the dose and dilution employed by Morrison, *et al.* In the 2 animals tested, this dye was secreted in the gastric juice within 3 minutes after the injection and increased in concentration for the duration of the experiment. Further observations upon basic dyes were considered unnecessary, in view of previous studies.[2, 3, 4]

The disagreement in the observed findings presented here and those of Morrison, *et al.*, may have one of several explanations. It is

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[5] Code, Charles F., and Varco, Richard L., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 475.

TABLE I.
Dye Excretion by Histamine-Excited Stomach Pouches.

| | | Acid M./Equiv./L. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Free | | Total | | | |
| Dog No. | Dye | Before | After Dye | Before | After Dye | Dye excretion | Histamine dosage and comment |
| 116 | Congo Red (1) | 140 | | | | 0 | 4 mg H. Subcut.: Depress. Vol. Secret. |
| 118 | ” ” (1) | 138 | | | | 0 | Same |
| 112 | ” ” (1) | 146 | | | | 0 | Same |
| 126 | ” ” (1) | 142 | | | | 0 | 20 mg H. in Beeswax Intra-musc. Depress. Vol. Secretion |
| 129 | ” ” (1) | 148 | | | | 0 | 20 mg H. in Beeswax Intra-musc. Depress. |
| 37 | ” ” (1) | 120 | | | | 0 | 6 mg H. Subcut.: Depress. Vol. Secret. |
| 129 | ” ” (1) | 128 | | | | 0 | 6 mg H. Subcut.: Depress. Vol. Secret. Reduct. Free Acid 128-70 M./Equiv./L. |
| 1 | Orange G (2) | 146 | 138 | 154 | 148 | 0 | 10 mg H. in Beeswax Intra-musc. |
| 1 | Mercurochrome (2) | | 105 | | 122 | 0 | Repeated Emesis after Inject. of Mercurochrome |
| 2 | Erythrosine (2) | 150 | 138 | 158 | 142 | 0 | 10 mg H. in Beeswax Inject. Intra-musc. |
| 2 | Phloxine (2) | | 156 | | 162 | 0 | |
| 3 | ” (2) | 116 | 80 | 130 | 96 | 0 | Same |
| 3 | Erythrosine (2) | | 70 | | 110 | 0 | |
| 4 | Mercurochrome (2) | 115 | 95 | 130 | 115 | 0 | 10 mg H. in Beeswax Intra-musc. Emesis after inj. of dye. |
| 4 | Orange G (2) | | 100 | | 133 | 0 | |
| 92 | Rose Bengal (2) | 148 | 152 | 156 | 158 | 0 | 12 mg H. in Beeswax injected intra-musc. |
| 92 | Lithium Carmine (2) | | 134 | | 142 | 0 | |
| 116 | Trypan Red (2) | 162 | 158 | 166 | 166 | 0 | 4.5 mg H. Subcut. |
| 116 | Rose Bengal (2) | | 146 | | 152 | 0 | |
| 126 | Eosin (2) | 140 | 106 | 150 | 116 | 0 | Same |
| 126 | Trypan Red (2) | * | | | | | |
| 129 | Lithium Carmine (2) | 137 | 132 | 147 | 142 | 0 | 12 mg H. in Beeswax Intra-musc. |
| 129 | Eosin (2) | | 125 | | 150 | 0 | |
| 92 | Chrysoidin (3) | 132 | 134 | 140 | 142 | + 3 min. | |
| 37 | ” (3) | 122 | | 133 | | + 3 min. | |

(1) Acid Colloidal Dye.
(2) Acid Dye.
(3) Basic Dye.
*Major portion of sample lost—no dye noted.

possible that there are differences in the chemical purity of the dyes used in the two studies. However, it seems more likely that another interpretation is correct. Morrison, *et al.,* found the colloidal dye Congo Red in pouch fluids. This dye is used for plasma volume determinations because it leaves the blood stream very slowly. We suggest that it may have appeared in pouch secretions either in serous exudate or in blood, more probably the former. The finding of a colloidal component of the plasma is most plausibly accounted for on such an assumption. We repeated the experiment on 7 dogs with 2 samples of Congo Red of different origins in order to be quite certain of a failure of excretion. If the interpretation given above is correct, the finding of other non-colloidal acid dyes in pouch fluid by Morrison, *et al.,* loses its theoretical significance. Our failure to find any of these dyes in highly acid secretions confirms this view.

*Summary.* A list of acid dyes reported to be secreted in gastric juice has been reinvestigated. None of them appeared in histamine-excited gastric juice from Pavlov or Heidenhain pouches in dogs. This work gives support to the earlier generalization that only dyes in which the chromogen may be in the cation, appear in highly acid gastric secretions.

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### 11973

## Peptic Activity of Achlorhydric Human Gastric Juices from Carcinomatous Stomachs. Comparative Study.*

Richard A. Rasmussen and Alexander Brunschwig.

*From the Department of Surgery and Medicine, the University of Chicago.*

A comparative study of the peptic activity of human gastric juices was made as part of a general investigation in the problem of the physiology of the cancerous stomach. It has been stated that achlorhydric juices from such stomachs are weaker in peptic activity than juices containing normal concentrations of free HCl, and that in this respect they resemble achlorhydric juices in general. In the recent literature, at least, there is but little specific data upon this question. Polland and Bloomfield[1] recorded their observations upon

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* This work was conducted under a grant from The International Cancer Research Foundation, Philadelphia, Pennsylvania.

[1] Polland, W. S., and Bloomfield, A. L., *Bull. Johns Hopkins Hosp.,* 1930, **46**, 307.

5 patients with histamine achlorhydria and gastric cancer, noting the weak peptic activity as determined by the edestin method devised by them.

While very accurate methods for the estimation of peptic activity have been described,[2] the classical Mett's tubes were employed in this study because of simplicity and because significance was to be attached only to gross differences, which this method would detect. Glass tubes of uniform bore, measuring 1.0 mm in diameter were employed, and egg albumin was drawn into them by suction. They were placed in boiling water until coagulation of the protein was apparent. They were then filed into segments about 2 cm in length and stored in distilled water at room temperature.

All samples of gastric juice were obtained from empty stomachs after over night starvation by the usual histamine clinical test for gastric secretory function.

The tests were conducted as follows: Five cc of the juice to be tested was brought, if necessary, to pH 1.2 (thymol blue as indicator) and placed in a 30 cc pyrex beaker. Two Mett's tubes were placed in the solution which was then placed in an incubator at 37.5°C for 24 hours, under watchglass covers. Normal HCl was used to adjust pH, thus the volumes of the various samples tested were practically the same. To further insure uniform conditions the same 2 beakers were employed throughout the study.

The results were recorded in terms of millimeters of albumin digested at each end of the tube; since 2 tubes were placed in each sample, four readings were obtained in each test; the final figure recorded for the test was the average of the 4 readings.

The results are summarized in Table I.

It is seen that achlorhydric juices contain substantially less pepsin

TABLE I.

| Source of juice | No. of different samples tested | Mm of Mett's tubes digested | Extreme Measurements in each series, mm |
|---|---|---|---|
| Patients with acid gastric juice presenting a variety of conditions | 36 | 6.1 | 3 to 9 |
| Carcinomatous stomachs secreting free acid | 5 | 5.9 | 4 to 8 |
| Pernicious anemia (achlorhydria) | 9 | 1.3 | 0 to 4 |
| Achlorhydria induced X-radiation to stomach | 9 | 1.6 | 0 to 4.75 |
| Achlorhydria without P.A., X-ray or gastric cancer | 12 | 1.7 | 0 to 6 |
| Achlorhydria with gastric cancer | 9 | 1.4 | 0 to 2.75 |

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[2] Gilman, A., and Cowgill, G. R., *J. Biol. Chem.*, 1930, **88**, 743.

than those containing free HCl. It is also demonstrated that in this series the low pepsin content of achlorhydric gastric juices from cancerous stomachs was of the same order as that observed in achlorhydric juices from other sources.

*Conclusions.* The low pepsin content of achlorhydric gastric juices from carcinomatous stomachs is of the same order as the low pepsin content of achlorhydric gastric juices obtained from patients not presenting carcinoma of the stomach.

## 11974

## Soluble Antigen in Lymphogranuloma Venereum.

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Neither the fundamental mechanism of the Frei reaction, which until recently was the only basis for the certain diagnosis of lymphogranuloma venereum, nor the nature of the reagent in the antigen which provokes a positive Frei test has yet been clearly defined. It has been observed that high-speed centrifugation of certain Frei antigens yields dermally active sediments containing virus elementary bodies[1] but nonreactive supernatant fluids.[1, 2] Furthermore, the passage of Frei antigens through Berkefeld filters[1, 2] or Seitz K discs[1] was found to reduce greatly or, more commonly, to abolish completely the dermal activity of the filtrate; it should be noted that Seitz K filters are permeable even to bacteria. These observations have led Findlay to the conclusion that the Frei test demonstrates an allergic reaction due to the presence of the elementary bodies of the virus in the material injected.

It has previously been shown that by propagation of the agent of lymphogranuloma venereum in the yolk-sac of the developing chicken embryo[3] or in the lungs of mice[4] there are obtained quantities of virus far greater than those present in other tissues. Inactivated suspensions of elementary bodies derived from the yolk-sac, and separated from tissue constituents by differential centrifugation, have been successfully employed as antigens for the Frei test[5] and for the demonstration of complement-fixing properties in the serum of persons infected with the virus;[6] in the latter test suspensions of virus from mouse-lung have also been used.

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[1] Findlay, G. M., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1938, **31**, 587.

[2] Sullivan, M., and Ecker, E. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1024.

[3] Rake, G., McKee, C. M., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

[4] Shaffer, M. F., Rake, G., and McKee, C. M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 408.

Complement-fixation tests[6] have been performed in which falling dilutions of selected lymphogranulomatous human sera, known to fix strongly, were titrated against falling dilutions of antigen in the form of suspensions of elementary bodies sedimented from yolk-sac or mouse-lung by high-speed centrifugation. From the results it appeared that with increasing dilution of either reagent, greater concentrations of the other were required for fixation of a constant dose of complement. By holding the serum dilution constant it was possible to titrate simultaneously several antigens for their relative fixing potency.

Using this technic, further experiments showed clearly that the complement-fixing properties, found to be associated with the virus-containing sediment deposited at 12 000 rpm, were also demonstrable in the corresponding supernatant fluid which previously had been shown to be largely depleted of live virus.[3] In Table I are summarized results obtained with single or pooled suspensions of 3 different strains of the virus, propagated in the yolk-sac or mouse-lung. It may be seen that, while the complement-fixing titer of the virus sediment, resuspended to the original volume of the 10% tissue suspension and then further diluted with saline, varied somewhat in the different experiments, in each case except Experiment 4 the corresponding high-speed supernate showed a relatively high degree of activity even after passage through Seitz EK filter. This was true for both yolk-sac and mouse-lung suspensions. A sample of the Seitz filtered supernate described in Experiment 5 of Table I was sent to Dr. A. W. Grace of the New York Hospital, who found that in known lymphogranulomatous persons it produced a positive Frei reaction in dilutions through 1:320 (highest tested), while controls with normal yolk-sac antigen were negative in these individuals.

These observations, so markedly at variance with the findings of earlier workers, made it desirable to determine whether the considerable activity of Seitz EK filtrates was due to a residuum of virus

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[5] Grace, A. W., Rake, G., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 259.

[6] McKee, C. M., Rake, G., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 410.

TABLE I.
Comparative Complement-Fixing Activity of Lymphogranuloma Virus Sedimented at High Speed* and Supernate Therefrom.

| Exp. No. | Strain of virus | Source of virus | Material tested | Complement-fixing titer† |
|---|---|---|---|---|
| 1 | NH | Yolk-sac | Resuspended high-speed sediment | 1:640 |
| | | | Unfiltered ” ” supernate | 1:320 |
| | | | Seitz-filtered ” ” ” | 1:80 |
| 2 | NH | Mouse-lung | Resuspended ” ” sediment | 1:160 |
| | | | Unfiltered ” ” supernate | 1:80 |
| | | | Seitz-filtered ” ” ” | 1:80 |
| 3 | JH | Yolk-sac | Resuspended ” ” sediment | 1:160 |
| | | | Seitz-filtered ” ” supernate | 1:40 |
| 4 | G | Yolk-sac | Resuspended ” ” sediment | 1:100 |
| | | | Seitz-filtered ” ” supernate | 1:10 |
| 5 | NH + JH + G | Yolk-sac | Resuspended ” ” sediment | 1:150 |
| | | | Seitz-filtered ” ” supernate | 1:50 |
| 6 | JH + G | Yolk-sac | Resuspended ” ” sediment | 1:150 |
| | | | Seitz-filtered ” ” supernate | 1:50 |

*The sediments and corresponding supernates were obtained by centrifugation at 12,000 RPM in the cold.

†The complement fixation tests were performed in Exp. Nos. 1, 2, 3 and 6 with serum K diluted 1:25, in Exp. No. 4 with serum LV No. 4 (of equal potency) diluted 1:25, in Exp. No. 5 with serum Z (more potent) diluted 1:50.

which had traversed the filters. Accordingly, several experiments performed in which suspensions of heavily infected yolk-sacs were centrifuged for an hour at 2000 to 3000 rpm in the cold to throw down gross particles. A portion of the supernate was passed through a Seitz EK disc under negative pressure not exceeding 65 cm mercury. Filtrate, as well as unfiltered suspension, was then tested not only for relative complement-fixing titer but also for infectivity by yolk-sac inoculation of the chicken embryo, which is by far the most delicate known indicator of minute amounts of active lymphogranuloma virus. The results are given in Table II, where it will be seen that passage through Seitz EK filters reduced the infectivity of the suspensions by one- to ten-millionfold or more; in all but one case, no virus whatever was detectable when a total of several cc of undiluted filtrate was inoculated into eggs. Nevertheless, complement-fixing potency of the filtrates remained one-eighth to one fiftieth that of the unfiltered material, values comparable to those obtained with Seitz EK filtrates of high-speed supernates (Table I) when possible clogging of the filters with material in these suspensions, which have not been centrifuged at high speed, is taken into consideration. The Seitz EK filtrate in Experiment D of Table II was also

TABLE II.
Infectivity and Complement-Fixing Potency of Lymphogranuloma Virus Yolk-Sac Suspensions Before and After Seitz EK Filtration.

| Exp. | Strain of virus | Material tested | Infectivity titer | Complement-fixing titer* |
|---|---|---|---|---|
| A | NH | Unfiltered suspension | $10^{-7}$ | 1:75 |
| | | Seitz EK filtrate | $<10^{-1}$ | 1:10 |
| B | NH | Unfiltered suspension | $2 \times 10^{-7}$ | 1:480† |
| | | Seitz EK filtrate | $< 2 \times 10^{-1}$ | 1:10 |
| C | JH | Unfiltered suspension | $10^{-8}$ | 1:75 |
| | | Seitz EK filtrate | $10^{-1}$ | 1:10 |
| D | JH | Unfiltered suspension | $5 \times 10^{-9}$ | 1:400 |
| | | Seitz EK filtrate | $< 5 \times 10^{-2}$ | 1:20 |

*The complement fixation tests were performed in Exp. A and B with serum Z diluted 1:50, in Exp. C with serum Z 1:100, in Exp. D with serum W 1:10.

†The period of fixation in Exp. B was longer than that usually employed.

submitted to Dr. A. W. Grace. In the highest dilution tested (1:120) it gave a strongly positive Frei response in known reactors who were at the same time negative to the control filtrate of normal yolk-sac.

The combined series of experiment shows clearly that the ability of Seitz EK filtrates, derived from rich suspensions of lymphogranuloma venereum virus, to fix complement specifically in the presence of lymphogranulomatous serum and to elicit a positive Frei reaction is due not to elementary bodies of the virus but to antigen in a less highly organized, possibly soluble, state. The failure of investigators to obtain similar results is doubtless due to the low concentrations of virus present in the materials which they employed.

### 11975

## Nature of the Capillary Permeability Factor Present in Extracts of Normal Tissues.

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Menkin[1] has shown that inflammatory exudates of various kinds have the property of increasing capillary permeability as demonstrated by the so-called trypan blue test in rabbits. This consists in the localization and concentration of intravenously injected trypan blue at the site of an intradermal injection of the exudate or preparations thereof. He ascribes this property to a specific substance in the exudates which he terms leukotaxine, and which, presumably, is liberated into the exudate from the injured tissues. Bier and Planet,[2] as well as Rigdon,[3] have reported that extracts of normal tissues exhibit this capillary permeability effect. Since Rocha e Silva and Bier[4] have shown that suitable concentrations of histamine give a positive trypan blue test, and since Bier and Planet found that extracts of rabbit's skin, which is comparatively rich in histamine, gave a positive test, while extracts of other rabbit tissues, which are poor in histamine, gave negative tests, it appeared probable that the capillary permeability factor in normal tissues might be histamine rather than some unknown specific tissue constituent. To test this possibility further, extracts from a variety of tissues have been examined with respect to their ability to give a trypan blue test and with respect to their histamine content.

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* Guggenheim Fellow, Instituto Biologico, Sao Paulo, Brazil.

[1] Menkin, V., *Physiol. Rev.*, 1938, **18**, 366.

*Method.* Simple extracts were prepared by grinding 2 to 4 grams of tissue with sand in saline. The amount of saline was varied in different experiments, so that extracts of varying activity could be studied. The extracts were filtered and assayed for their histamine-equivalent content, using the isolated atropinized intestinal strip of the guinea pig for the assay. A solution of histamine acid phosphate of the same activity as the extract was prepared and both extract and histamine solution were injected into the abdominal skin of previously shaved rabbits. A few minutes after the intradermal injections, trypan blue (approximately 10 cc of 1%) was injected via an ear vein. The intensity of coloration of the respective areas was compared at intervals up to 30 minutes after the trypan blue injection.

The results in 7 experiments with guinea pig tissues are shown in Table I and it will be seen that in general the ability of an extract to produce a trypan blue reaction can be accounted for by the histamine activity of the extract, as determined by its effect on the atropinized guinea pig intestinal strip. Trethewie[5] has shown that the histamine activity of a saline extract of tissues is frequently increased by heating due to the solution of suspended incompletely fragmented cells. The above extracts were therefore boiled, reassayed as to their histamine activity and retested as to their permeability effect. In

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[2] Bier, O., and Planet, U., *Compt. rend. Soc. Biol.*, 1938, **129**, 65.

[3] Rigdon, R. A., *Arch. Surg.*, 1940, **41**, 96.

[4] Rocha e Silva, M., and Bier, O., *Arq. Inst. Biol.*, 1939, **19**, 219.

[5] Trethewie, E. R., *Austr. J. Exp. Biol. and Med. Sci.*, 1938, **16**, 225.

TABLE I.
Comparative Trypan Blue Tests Produced by Extracts of Guinea Pig Tissues and by Solutions of Histamine.

| | | Histamine* equivalent | Trypan blue test |
|---|---|---|---|
| I. | Lung | 2.5 | +++ |
| | Histamine | 2.5 | +++ |
| II. | Lung | 3.0 | +++ |
| | Liver | 0.45 | 0 |
| | Muscle | 0.18 | 0 |
| | Histamine | 3.0 | +++ |
| III. | Lung | 2.5 | +++ |
| | Liver | — | 0 |
| | Histamine | 2.5 | +++ |
| IV. | Lung | 3.3 | +++ |
| | Histamine | 3.3 | +++ |
| V. | Lung | 10.0 | ++++ |
| | Liver | 2.5 | +++ |
| | Histamine | 10.0 | ++++ |
| VI. | Lung | 4.0 | ++++ |
| | Liver | 0.4 | + |
| | Muscle | 0.07 | 0 |
| | Histamine | 4.0 | +++ |
| VII. | Lung | 15.0 | ++++ |
| | Liver | 1.5 | + |
| | Muscle | 0.5 | 0 |
| | Histamine | 15.0 | +++ |

*Micrograms of histamine acid phosphate per cc.

every instance in which the histamine activity increased significantly, the trypan blue reaction likewise increased.

It is well known that, in contradistinction to the guinea pig, the liver of the dog is rich in histamine. Consequently saline extracts of dog's liver, lung, and muscle, were tested as above. The liver extracts gave markedly positive trypan blue reactions, the muscle extracts were negative and the lung extracts were active but less so than the liver. Similarly rabbit's blood contains a large amount of histamine in comparison with the blood of guinea pigs or dogs. Serum, obtained from freshly clotted whole blood of rabbits, guinea pigs, and dogs, was studied. Rabbit's serum gave markedly positive trypan blue reactions, guinea pig serum was usually negative, while dog's serum gave a mild reaction. The latter could not be accounted for by its histamine-equivalent content. Extracts were made by Code's method[6] from the whole blood of a number of rabbits. These extracts produced trypan blue reactions, indicating that the capillary permeability factor can survive boiling with concentrated hydrochloric acid.

It is clear, therefore, that the ability of tissue extracts to produce a positive trypan blue test in rabbits can, in most instances, be accounted for by their histamine activity. The reason for the occasional slight discrepancy is not apparent. Duthie and Chain,[7] however, have shown that protein degradation products obtained from a variety of proteins by a variety of proteolytic enzymes, can elicit a trypan blue reaction. It is not surprising, therefore, that tissue extracts may occasionally manifest an activity of this sort which cannot be accounted for by their histamine content. There seems no necessity, therefore, for postulating a specific factor in tissues which has the property of increasing capillary permeability as evidenced by the trypan blue reaction.

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[6] Code, C. F., *J. Physiol.*, 1937, **89**, 257.

[7] Duthie, E. S., and Chain, E., *Brit. J. Exp. Path.*, 1939, **20**, 417.

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### 11976

## Rapid Response of Guinea Pig Thyroid to a Single Injection of Thyrotropic Hormone.

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Microhistrometric studies[1] of the guinea pig thyroid after 3 daily subcutaneous injections of thyrotropic hormone have been published. It had been assumed that 3 or 4 days were required for significant histologic change to occur. Loeb[2] had noted early hypertrophy one day after an intravenous injection. Aron[3] also reported that changes were evident in less than 12 hours. Janssen and Loeser[4] described structural changes in 2 hours after a single injection of 20 guinea pig units. Kummer[5] also described changes apparent at 2 hours which were more pronounced at 4 hours. Williams,[6] by a method of continuous *in vivo* observation, detected a cycle of activity that reached a maximum within 24 hours. Okkels,[7] in his classical monograph of 1934, states "that 30 minutes after intraperitoneal injection in the guinea pig of Evans' Pituitary Extract structural changes appear in the thyroid cells. The colloid is not yet affected but the thyroid cells develop a tremendous swelling, presumably like a gel-sol transformation of the cytoplasm." A chronological and quantitative representation of the changes occurring in the guinea pig thyroid after a single subcutaneous injection of the thyrotropic hormone is desirable.

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[1] Rawson, R. W., and Starr, Paul, *Arch. Int. Med.*, 1938, **61**, 726.

[2] Loeb, L., and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 490.

[3] Aron, M., *Rev. Frac. d'Endocrinol.*, 1930, **8**, 472.

[4] Janssen, S., and Loeser, A., *Arch. f. Exp. Path. u. Pharmakol.*, 1931, **163**, 517.

[5] Kummer, H. J., *Endokrinologie*, 1938, **20**, 326.

[6] Williams, R. G., *Am. J. Anat.*, 1937, **62**, 1.

[7] Okkels, H., *Skan. Arch. f. Phys.*, 1934, **69**, 97.

*Method.* One hundred seventeen young, 200-300 g male guinea pigs were used; of these 26 served as controls. Male animals were used to obviate any effect of the estrous cycle upon the thyroid. In order to standardize the conditions of the experiment as much as seemed feasible, all of the experimental animals were submitted simultaneously to a single subcutaneous injection of 0.03 cc of Antuitrin-T,* and groups of animals—both experimental and control—were subsequently sacrificed at short predetermined incremental intervals. The chronological periods subsequent to injection, and number of animals in each group were as follows: 1 hour, 8 animals; 4 hours, 19 animals; 6 hours, 12 animals; 8, 12, 16, 20, and 24 hours, 8 animals each; 32 and 48 hours, 6 animals each. Their thyroid glands were quickly but carefully removed, preserved in formalin, embedded in paraffin, stained with hematoxylin and eosin, and prepared for histological examination.

Utilizing the method of Rawson and Starr—which simply involves the accurate measuring and recording of the height of a typical epithelial cell in 100 acini, from which measurements the arithmetical mean acinar cell height is determined—a quantitative record of the changes in mean acinar cell height at chronological intervals during the 48 hours subsequent to a single injection was obtained.

*Results.* Of the 26 control animals the average mean acinar cell height was found to be 4.45 micra with a range of plus or minus 1.5 micra. The mean acinar cell heights of groups of animals killed at 1, 4, 6, 8, 12, 16, 20, 24, 32, and 48 hours after injection were found to be 5.13, 5.34, 5.37, 7.57, 7.74, 8.44, 7.86, 8.49, 7.92, 7.58, respectively. The most striking change observed was an enormous increase in cell height by 8 hours; the maximum response in this series was obtained at 16 hours, the acinar epithelium nearly doubling in height; and the mean acinar cell height was still markedly increased over the control value at 48 hours. (Fig. 1.) The trend of the graph is corroborated by statistical analyses; for since the significant critical ratio is 2, it is evident that all increases in mean acinar cell height including and subsequent to the 4-hour period are statistically significant; that the hypertrophy is marked at 8 hours, maximal at 16 hours, and maintained at least 48 hours following a single subcutaneous injection of thyrotropic hormone.

*Discussion.* In the interpretation of the experimental response, the individual variability of the animals utilized was considered. Prior to the 8-hour period, not all animals responded significantly; however, at 8 hours and thereafter all values obtained are well above

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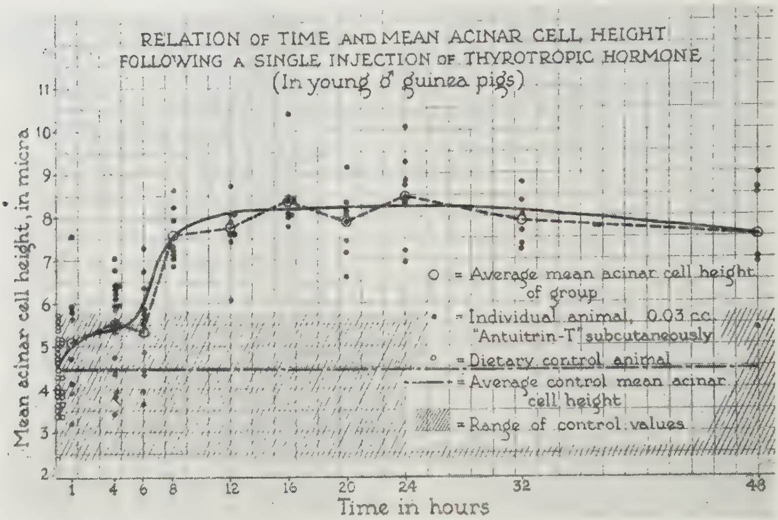
* Generously supplied by Dr. E. A. Sharp, Parke Davis and Company.



the range of the control group. Reference to the graph indicates that some animals respond more quickly, others more tardily; that some maintain the response to stimulation for prolonged periods subsequent to injection, while in others recession occurs more promptly.

The relation between mean acinar cell height and dosage of Antuitrin-T was determined,[8] and it was found that the maximal mean acinar cell height was attained with a 0.03 cc of Antuitrin-T subcutaneously, further increase in dose having little effect. It was noted, too, that the group average obtained with that dose approximates 7.8 micra, while the maximum group response in the present series lies between 7.5 and 8.5 micra; however, that response was obtained after 3 daily subcutaneous injections, killing the animals on the fourth day; and it is evident that the same height of response may be attained in 8 to 16 hours following a single subcutaneous injection. Careful study of the amount, condition, and staining reaction of the colloid in these glands indicates that it underwent alterations associated with the changes in mean acinar cell height in response to stimulation.

*Summary and Conclusions.* A single, subcutaneous injection of thyrotropic hormone will result in a significant increase in mean acinar cell height within 8 to 16 hours, which is comparable to that heretofore reported in 72 hours following a series of daily injections. This measured response persists at least 48 hours following injection.

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[8] Starr, P., and Roskelley, R., *Am. J. Phys.*, 1940, **130**, 549.

## 11977

# Protective Action of Mercury and Lead Salts Against Procaine Convulsions.*

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Recently Beutner, Landay and Lieberman[1] reported that hydroxy mercuri-methoxy-propyl carbamyl phenoxy acetate (salyrgan) will act as an anticonvulsant drug. They found that salyrgan prevented the usual procaine convulsions if the two drugs are mixed prior to the injection. They also noted that if the salyrgan is injected separately before that of procaine, it afforded no protective action against the procaine. They concluded from these experiments that the "anticonvulsant" action of salyrgan is not through a renal diuretic action but rather by virtue of a "tissue diuretic" action, *i. e.*, decreased cellular permeability.

A series of experiments was performed in our laboratory in which the following intramuscular injections were made into guinea pigs: (1) procaine alone, (2) procaine and salyrgan into separate areas of the body, (3) procaine and salyrgan mixed before the injection, (4) procaine and mercuric chloride mixed before the injection, and (5) lead nitrate and procaine mixed before the injection.

*Results.* Our experiments confirm Beutner, *et al.*, that salyrgan will protect an animal against procaine convulsions if the 2 drugs are mixed before being injected, but not if the 2 drugs are injected separately in different regions of the body. In 17 experiments performed on as many guinea pigs, 150 mg per kilo of procaine (10% solution) were injected intramuscularly into the right rear leg. Sixteen of the animals showed violent convulsions, from which they recovered in 15 to 40 minutes. In 12 experiments 20 mg per kilo of a 10% solution of salyrgan were injected intramuscularly and immediately following 150 mg of procaine per kilo were injected intramuscularly into the opposite extremity. All of these animals had convulsions which were as severe and lasted as long as those in which procaine was injected alone. In another series of 10 experiments the same quantities of procaine and salyrgan per kilo were

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* This investigation was made possible through a grant from Parke, Davis and Company to Jefferson Medical College for research in science.

[1] Beutner, R., Landay, J., and Lieberman, A., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 120.

mixed and injected intramuscularly. In not a single experiment did the animal have a convulsion.

In our experiments it was noted that when salyrgan and procaine are mixed a white precipitate is produced. In the quantities used in these experiments, *i. e.*, 20 mg salyrgan per 150 mg procaine, the precipitate does not persist but redissolves upon agitation. If, however, the quantity of salyrgan is increased to 75 mg per 150 mg of procaine, a permanent white precipitate is produced. It appears from this that salyrgan and procaine are incompatible, the mercury in the salyrgan molecule probably acting as an alkaloidal precipitant.

A series of similar experiments was performed on guinea pigs in which an inorganic mercury salt was substituted for the salyrgan. In 10 experiments in which 150 mg of procaine (10% solution) and 3 mg mercuric chloride (1% solution) per kilo were injected separately into different areas of the animal, convulsions were noted in every instance. However, in 31 experiments in which the same quantities of procaine and mercuric chloride were mixed prior to the injection, mild convulsions were noted in only 8 of the animals. It was noted also that small quantities of mercuric chloride when added to a 10% procaine solution produce a white precipitate, which redissolves upon agitation. Larger quantities, as with the salyrgan, will produce a permanent white mercury-procaine precipitate.

Lead nitrate was found to be nearly as effective as mercuric chloride in protecting against procaine convulsions. In 12 experiments 150 mg of procaine (10% solution) and 10 mg lead nitrate (5% solution) were mixed in a test tube and injected intramuscularly into guinea pigs. In these experiments only 5 of the 12 animals had convulsions.

From these experiments we conclude that salyrgan, mercury chloride and lead nitrate protect against procaine convulsions by virtue of their alkaloidal precipitating properties. The mercury-procaine and lead-procaine compounds are probably absorbed too slowly to produce convulsions. Intravenous injections of salyrgan-procaine and lead-procaine mixtures are equally as toxic as pure procaine solutions.

## 11978

## Effect of Sympathectomy upon Blood Supply of Bone.

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Elevation of skin temperature following sympathectomy is a matter of common observation. This has been shown to be due to vascular dilatation and increased circulation. Leriche and Policard,[1] Ferrannini,[2] Frugoni,[3] and Introzzi[4] have shown that, following interruption of sympathetic nerve supply to bone, some degree of decalcification occurs as evidenced by roentgenogram or ash analysis. They have assumed that this effect was due to increased blood supply to the bone. But, so far as we know, no evidence has been presented that any increase in the blood supply actually occurs. The purpose of this study is to determine the effect of sympathectomy upon the blood supply of bone

*Method and Material.* Previously Griffith, Zinn and Comroe[5] reported the effect of bilateral lumbar sympathectomy upon the vasa vasorum of rats. Carbon particles, in the form of ink, were injected into the blood stream and later identified in unstained sections of the femoral artery. It was found that lumbar sympathectomy increased the number of carbon particles in the outer coats and immediately about the adventitia of the femoral artery, and it was therefore believed that it had increased the number of open vasa vasorum. Twelve of the legs used in this study had been preserved, including those of 6 normal and 6 sympathectomized animals. All the tibiae were decalcified and prepared for histologic section. Sections were examined both stained and unstained and the carbon in the minute vessels of the bone noted.

*Results.* It was obvious that, in general, there was much more carbon in the bones of the normal animals than in bones of sympathectomized animals. The manner of expressing this difference was a problem. It was decided to arbitrarily grade the carbon concentra-

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* Atwater Kent Fellow in Medicine.

[1] Leriche, R., *Physiologie et pathologie du tissu osseux,* Paris, Masson et Cie, 1939.

[2] Ferrannini, A., *Pathologica,* 1935, **27**, 777.

[3] Frugoni, P., *Arch. di Biol.,* 1934, **88**, 27.

[4] Introzzi, A. S., *Semena Med.,* 1934, **1**, 1641.

[5] Griffith, J. Q., Jr., Zinn, C. J., and Comroe, B. I., *Arch. Path.,* 1938, **26**, 984.

TABLE I.
Incidence of Carbon in the Small Vessels of Bone in Normal and Sympathectomized Rats, in Order of Ascending Concentration.

| Amount of carbon | No. of animals | |
|---|---|---|
| | Normal | Sympathectomized |
| + | 0 | 1 |
| ++ | 1 | 2 |
| +++ | 1 | 3 |
| ++++ | 4 | 0 |

tion in the bones in 4 degrees. This grading has been designated in Table I by plus signs. Roughly, ++++ concentration is 4 times + concentration.

*Discussion.* In the previous study[5] it had been shown that more carbon was present in the vasa vasorum of the femoral artery of the sympathectomized animal. This was in accord with the general observation of increased blood supply to the skin after sympathectomy. The present findings in respect to bone appear to be contradictory to this trend. The following explanation is offered. Vascular dilatation may be said to occur if (1) already open vessels open wider, or (2) small vessels open that had previously been closed. Studies based on carbon injection show changes principally if the second circumstance applies, *i.e.*, if new vessels open. Thus it may be that in bones most or all of the vessels are constantly open, whereas in the skin less than half of the vessels are usually open at a single time. Even if some new vessels in the bone do open, the amount of blood reaching them might be diminished due to their sharing the common blood supply of the leg with more widely open vessels in the softer parts. This effect would probably be most marked the greater the dilatation of the vessels in the soft tissues. In this connection it should be stressed that the studies were made on the fifth day postoperatively, at which time dilatation is thought to be maximal. These relations might not hold at a later period.

*Conclusions.* On the fifth day following lumbar sympathectomy the blood supply to the tibia in the rat is decreased. This paradoxical effect may be the result of disproportionately greater dilatation of the vessels in soft tissues.

### 11979

# Position of the Carbon Dioxide-Carbon in Propionic Acid Synthesized by *Propionibacterium*.*

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The assimilation of carbon dioxide by heterotrophic bacteria was first established by Wood and Werkman[1] with the propionic acid bacteria. Recently the stable isotope of carbon $(C^{13})^2$ and radioactive carbon $(C^{11})^3$ have been used as tracers of the carbon dioxide fixed in the fermentation of glycerol. The fixed carbon has been shown to be predominantly in the products propionic and succinic acids. Wood, *et al.*,[2] demonstrated, in addition, that the fixed carbon in succinic acid is exclusively in the carboxyl group. These authors suggested that all the carbon dioxide is fixed by the following reaction

$$CO_2 + HCH_2 \cdot CO \cdot COOH \longrightarrow COOH \cdot CH_2 \cdot CO \cdot COOH$$

and that propionic acid containing fixed carbon is derived from the 4-carbon compounds. Accordingly, the fixed carbon of propionic acid would be in the carboxyl group. Therefore, the report by Carson, *et al.*,[4] of fixed carbon occurring in the $\alpha$- and $\beta$-carbons as well as in the carboxyl carbon of propionic acid is of particular interest. These results imply that, in addition to the mechanism shown by Wood, *et al.*,[2] the propionic acid bacteria have another method of fixing carbon dioxide. The latter method might involve the synthesis of the entire chain from 1-carbon compounds, since the fixed carbon is suggested by Carson, *et al.*,[4] to be distributed among the 3 carbons of propionic acid. This observation places in question the present schemes of fermentation, for the implication is that the substrate

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* Journal paper No. J-828 of the Iowa Agricultural Experiment Station. Project No. 572.

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[1] Wood, H. G., and Werkman, C. H., *J. Bact.*, 1935, **30**, 332; *Biochem. J.*, 1936, **30**, 618.

[2] Wood, H. G., Werkman, C. H., Hemingway, Allan, and Nier, A. O., *J. Biol. Chem.*, 1940, **135**, 789; 1941 submitted.

[3] Carson, S. F., and Ruben, S., *Proc. Nat. Acad. Sci.*, 1940, **26**, 422.

[4] Carson, S. F., Foster, J. W., Ruben, S., and Kamen, M. D., *Science*, 1940, **92**, 433.

may first be broken down to 1-carbon compounds and then synthesized to the final products of fermentation. While it is clear that autotrophic forms of life must perform such a synthesis, it does not seem necessary for heterotrophic forms of life to break down the substrate to a 1-carbon compound and then resynthesize it to final products. We have, therefore, reinvestigated the problem. Contrary to the report of Carson, *et al.*,[1] we find the fixed carbon is exclusively in the carboxyl group of propionic acid.

The experimental procedure was as follows: Propionic acid containing fixed carbon enriched with $C^{13}$ was obtained from the fermentation of glycerol as previously described.[2] The volatile acids in the steam distillate contained approximately 3.0 g of propionic acid. The acids were neutralized, evaporated to 5 ml, acidified with 10 N sulphuric acid and the mixture was extracted three times with 5 ml portions of ether. The ether solution was dehydrated with calcium chloride and then distilled in a small fractionating column. The fraction containing propionic acid (136-141°C, mostly at 140°C) weighed 2.1 g and was degraded by the following reactions:

$$CH_3 \cdot CH_2 \cdot COOH \xrightarrow{\text{P and Br, then } H_2O} CH_3 \cdot CHBr \cdot COOH$$

$$CH_3 \cdot CHBr \cdot COOH \xrightarrow{AgOH} CH_3 \cdot CHOH \cdot COOH$$

$$CH_3 \cdot CHOH \cdot COOH \xrightarrow{KMnO_4} CH_3CHO + CO_2$$

$$CH_3CHO \xrightarrow{I_2 \text{ (alkali)}} CHI_3 + HCOONa$$

The above conversion was carried out as follows:

The reaction mixture after α-bromination[5] was made alkaline to litmus with silver oxide and then acid with hydrochloric acid to precipitate the silver and give free lactic acid. The filtrate solution was oxidized with potassium permanganate[2] and the resulting aldehyde and carbon dioxide were collected in bisulfite and alkali respectively. The recovery was about 40% (11.2 mM of acetaldehyde determined by iodine-titration of bound sulfite and 12.0 mM of carbon dioxide). Part of the aldehyde solution was oxidized[6] to carbon dioxide for mass spectrographic determination[7] of its $C^{13}$ content. The remainder was converted into iodoform and formic acid.[8] The excess iodine in the iodoform reaction was titrated with thiosulfate and the mixture was then filtered. The iodoform was

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[5] Cohen, J. B., *Practical Organic Chemistry*, 1924, p. 110, Macmillan, London.

[6] Osburn, O. L., and Werkman, C. H., *Ind. Eng. Chem.*, Anal. Ed., 1932, **4**, 421.

[7] Nier, A. O., *Rev. Sci. Instruments*, 1940, **2**, 212.

[8] Goodwin, L. F., *J. Am. Chem. Soc.*, 1920, **42**, 39.

TABLE I.

| Fraction of propionic acid | % $C^{13}$ |
|---|---|
| Entire molecule | 1.35 |
| $\alpha$ and $\beta$-carbon ($CH_3CHO$) | 1.07 |
| $\alpha$-carbon (HCOONa) | 1.08 |
| $\beta$-carbon ($CHI_3$) | 1.08 |
| COOH-carbon ($CO_2$) | 1.77 |
| COOH-carbon (calculated value)* | 1.87 |

*$2 \times 1.09 + X = 3 \times 1.43$. $X = \%$ $C^{13}$ in COOH-carbon.

dried for 24 hours at room temperature over calcium chloride and then oxidized to carbon dioxide by dry combustion. Formic acid in the filtrate was removed by steam distillation and oxidized to carbon dioxide with mercuric oxide.[9] The formic acid and iodoform were formed in equimolar quantities but the yield was only 50% on the basis of the acetaldehyde. From 4.0 mM of acetaldehyde 2.0 mM of iodoform and 2.1 mM of carbon dioxide were obtained. The iodoform reaction with acetaldehyde is not quantitative.[10] However, since the iodoform and formic acid were formed in equimolar amounts, these compounds undoubtedly arise from the $\beta$ and $\alpha$ carbons respectively of the acetaldehyde. The data from the above procedure are given in Table I.

It is to be remembered in considering the data in Table I that all natural carbon contains approximately 1.09% $C^{13}$. The value 1.35 for propionic acid indicates there is carbon present in the molecule which was derived from fixed-carbon dioxide in which the $C^{13}$ content had been artificially increased to serve as a tracer. The experimental variation in the mass spectrographic analysis is about ±0.02. Therefore the value 1.35 is clearly indicative of fixed carbon dioxide. The $\alpha$ and $\beta$ carbons of the propionic acid contained the normal complement of $C^{13}$, evidently there was no fixed carbon in these positions. The carboxyl carbon, on the other hand, contained a concentration of $C^{13}$ above that of normal carbon. The fixed carbon from $C^{13}O_2$ was obviously present in this position. The agreement between the experimental and calculated values, on the basis that all the fixed carbon is contained in the carboxyl group substantiates this conclusion. This fact is in agreement with the authors' concept of the mechanism of carbon dioxide-fixation through synthesis with 3-carbon and 1-carbon compounds. The discrepancy between our results and those of Carson, *et al.*,[4] calls for further investigation.

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[9] Osburn, O. L., Wood, H. G., and Werkman, C. H., *Ind. Eng. Chem.*, Anal. Ed., 1933, **5**, 247.

[10] Hatcher, W. H., and Mueller, W. H., *Trans. Roy. Soc. Canada*, 1929, Ser. 3, **23**, Sect. 3, 35.

There are two possibilities, either the chemical reactions used are unreliable or the synthetic reactions of the bacteria differed under the respective experimental conditions. That the stepwise degradation used in our investigation was reliable is shown by the quantitative recovery of the $C^{13}$ in the carboxyl group. We are at present endeavoring to determine the reliability of the chemical reactions used by Carson, *et al.*,[4] by degrading chemically synthesized propionic acid containing $C^{13}$ in the carboxyl group.

The authors wish to express appreciation to Professor Henry Gilman and Mr. C. G. Stuckwisch for assistance in the degradation of the propionic acid.

### 11980

## Repeated Attacks of Influenza.

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Recent reports[1, 2, 3] have established the principle that illnesses which on the basis of their similar clinical symptoms would be classified together as influenza can be caused by antigenically different viruses. The same principle must be taken into account when repeated attacks of influenza occur in an individual; that is, in a person who previously has had influenza a subsequent attack may be due to infection with a virus that is antigenically different from the one responsible for the earlier illness. That examples of that sort do occur will be shown by the results of the following experiments in which samples of serum obtained over a period of 4 to 5 years from persons who had had more than one attack of influenza were tested against two antigenically different viruses, the *TM* virus[1, 3] and the *PR8* strain of the virus of epidemic influenza.[4]

Patient A had 2, and Patient B had 3 attacks of influenza during the period of observation. The serums, which were 14 from each patient, included samples obtained at the time of onset of the symp-

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[1] Magill, T. P., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 162.

[2] Francis, T., Jr., *Science*, 1940, **92**, 405.

[3] Magill, T. P., and Tyndall, M., in press.

[4] Francis, T., Jr., *Science*, 1934, **80**, 457.

toms of the illnesses, samples obtained 3 to 4 weeks later, and samples obtained at reasonably regular intervening intervals. In order to make the comparison valid all of the samples from each patient were tested at the same time. Each serum dilution was mixed with an equal volume of a suspension (mouse lung) which contained between 100 and 1000 lethal doses of the respective virus per 0.03 cc, and after incubation at 37°C for ½ hour 0.06 cc of the mixture was given intranasally to mice which were under ether anesthesia. The

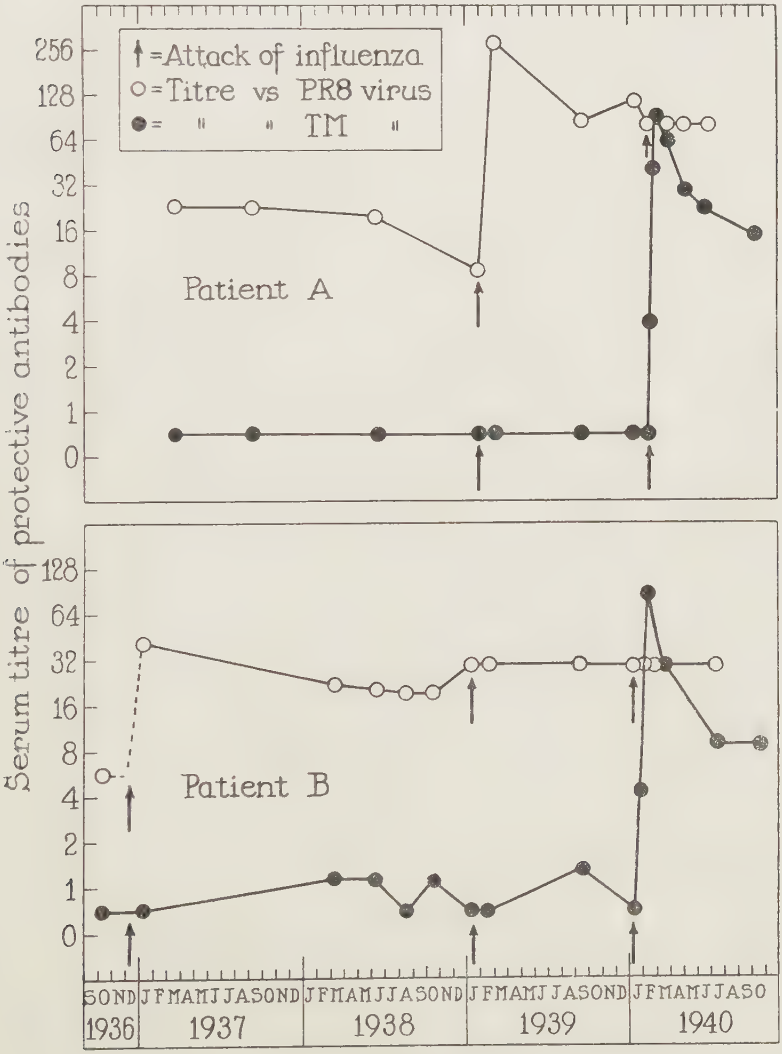

FIG. 1.

tests were terminated on the tenth day and the serum antibody titres were calculated as the dilution of serum that would protect 50% of the mice from death.[5] The results are summarized in Fig. 1 in which the serum antibody titers against the 2 viruses are plotted against the time at which the respective serum had been obtained; the serum titers are expressed as the actual dilution of serum before the addition of the virus suspension. The time of onset of each attack of influenza is indicated by an arrow.

The data (Fig. 1) show that as measured by the capacities of the serums to protect against the two viruses, the different attacks of influenza induced different antibody responses. Patient A had influenza twice: the January, 1939, illness evoked antibodies against the *PR8* but not against the *TM* while, in contrast, the January, 1940, illness evoked antibodies against *TM* but not against *PR8*. Patient B had influenza 3 times: the antibody responses to his 1936 and 1940 illnesses were similar respectively to those of Patient A's 1939 and 1940 attacks; but his 1939 attack differed from any of the others observed with him or with Patient A in that it did not cause a rise in antibodies against either the *TM* or the *PR8* viruses.

On the basis that each infection should be followed by a significant increase in antibodies against the specific causative agent or against agents closely related to it, these data would be interpreted as follows: Patient A's 1939 and B's 1936 illnesses were due to a virus identical with or closely related to the *PR8*; both Patient A's and Patient B's 1940 illnesses were due to a virus identical with or closely related to the *TM*; whereas Patient B's 1939 illness was due to some virus not closely related to either the *TM* or the *PR8*. If those deductions are correct the results presented in this paper furnish convincing examples of instances in which the subsequent attacks of a person who previously had had influenza may be explained on the basis of antigenic differences in the infecting agents.

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[5] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

## 11981

# Further Experiments with Poliomyelitis Virus in Cotton Rats.*

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It has been shown that Flexner's M.V. poliomyelitis strain carried in monkeys can be acclimated to rats[1] and subsequently retransferred from rats to monkeys, in which latter typical disease was produced.[2] The first 2 experiments of this report were performed in order to determine whether the virus could be retransferred from the collateral monkey generation back to rats.

*Experiment I.* Monkey No. 2379 was 4 generations removed from the original rat transfer. Generation I monkey had received intracerebrally 1 cc of a 10% lineal generation VI cotton rat cord virus. Generations II, III, and IV monkeys were given 1 cc of a 10% cord virus obtained from the generations I, II, and III monkeys, respectively.

*Generation I Rat Retransfer.* On 11/11/40, 5 cotton rats were injected with 0.06 cc IN, 0.06 cc IC, 0.5 cc SQ, and 1 cc IP of a 10% cord virus suspension of generation IV, Monkey No. 2379. (The doses mentioned here are the same in all the subsequent experiments of this type.)

Two animals died—1 on 11/27, the sixth day, and 1 on 11/28, the seventh day; both were weak, but neither had paralysis.

*Generation II Rat Retransfer.* On 11/29/40, the cords of the 2 animals dying in rat generation I were injected into 3 cotton rats. One rat died on 12/1 with no evidence of paralysis; the other 2 animals had paralyses and died on 12/5.

*Generation III Rat Retransfer.* On 12/6/40, a cord suspension made from the 2 animals that died on 12/5 in rat generation II was injected into 3 animals, all of which developed paralysis.

Thus, one can transfer Flexner's M.V. strain from the monkey to the cotton rat, back to the monkey and then to the rat again. It will be noted that the animals dying in generation I rat retransfer did not have typical paralysis. Typical paralysis never has been seen by us

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* Sponsored by The National Foundation for Infantile Paralysis, Inc.

[1] Toomey, John A., and Takacs, William S., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 364.

[2] Toomey, John A., and Takacs, William S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 22.

in a generation I rat transfer. In these experiments, "filtrate" was not necessary to initiate the transfer.

*Experiment II.* It was thought that a transfer from a rat cord to a generation I monkey might leave enough of "rat specificity" in the acclimated animal to produce immediate paralysis if this monkey cord, only one generation removed from the rat, were transferred back to the rat. Monkey No. 2387 was a first generation take from generation XI rat cord virus. This animal, injected 11/8/40, had quadriplegia on 11/16.

The monkey cord virus was injected into 2 cotton rats on 11/19, 1 of which had weakness and died 11/27. Three other animals were injected on 11/25, 1 of which had weakness and died on 12/3. Three animals lived.

*Generation II.* A suspension of the cord and brain of the first animal dying in generation I was injected into 3 cotton rats on 11/29/40. All had weakness; 1 rat died on 12/10, 1 on 12/16 and 1 on 12/20.

*Generation III.* A combination suspension of the cord and brain of the animals in generation II that died on 12/10 and 12/16 was injected into 5 animals on 1/2/41. One animal died accidentally after the injection. The 4 remaining animals had paralysis; 1 on 1/14, 2 on 1/16, and 1 on 1/18.

Thus, it was again possible to transfer Flexner's M.V. strain from monkeys to rats, to monkeys, and back to rats. It would seem, however, that heterologous monkey virus, even though but one generation removed from the parent rat stock, must again become acclimated to the rat and that 2 or 3 transfer generations are necessary before paralysis can be produced.

*Experiment III.* The third experiment was done in order to determine whether or not we were dealing with a true poliomyelitis virus in these experiments. A 4% suspension of Flexner's M.V. strain of acclimated generation XV rat cord virus was made. To different lots of this suspension were added equal quantities of: (a) horse serum obtained from an animal which lacked any virucidal antibodies as proven by many previous neutralization experiments in monkeys;[3] (b) horse serum obtained from the same animal after it had undergone a long series of injections with Flexner's M.V. virus, fortified with enteric organisms and toxins;[3] a serum which was found highly virucidal in previous neutralization experiments in monkeys; (c) pooled convalescent poliomyelitis human serum; (d) saline. One group of rats received convalescent serum only and, another, horse serum only (plus saline in each instance to make up a comparable dosage). Serums, saline, etc., were added to the virus mixture as indicated, the combination shaken, incubated for 1 hour and placed in the icebox for 24 hours, warmed and injected as follows: 0.06 cc IC, 0.06 cc IN and 0.5 cc SQ. The final injection mixture was a 2% suspension of virus.

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[3] Toomey, John A., *Am. J. Dis. Child.*, 1937, **53**, 1492.

Protection may be said to have been demonstrated if the animals which received the neutralized virus lived at least 21 days without having paralysis after the test was done.

Every one of the positive controls developed paralysis and died within 10 days after the injection. All of the animals, injected with the horse serum lacking any neutralizing antibody titer, also died. None of the animals injected with either the normal pooled convalescent serum or specific horse serum died or developed disease. In the pooled human convalescent serum protected group, 4 of the 20 animals died, a 20% mortality rate. Of the 20 animals injected with the specific neutralizing horse serum and virus, 1 was killed by other rats the first day after the injection; of the 19 that remained, 1 died without signs of paralysis on the thirteenth day. Only one of the 19 died, a 5+% mortality rate as compared with that of 100% following the injection of serum obtained from the same horse prior to immunization.

These specific neutralization tests demonstrate that the virus material transferred to the cotton rat was truly a poliomyelitis virus strain. Since the original acclimations to the cotton rat were made with Flexner's M.V. strain, it can be assumed that the poliomyelitis strain which had been carried in the rat was the Flexner's M.V. strain.

### 11982

## Salt After Adrenalectomy. IV. Urinary Nitrogen and Survival Time of the Fasted Adrenalectomized Salt-treated Rat.*

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Adrenalectomized rats kept under optimal living conditions and allowed to drink 1% NaCl solution store fed glucose as liver glycogen

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almost as well as intact animals. They continue to grow and to maintain a satisfactory state of health; animals so treated have been observed for periods as long as 176 days postoperative.[1] In this study such animals were fasted and the urinary nitrogen and survival time noted.

Our first observations were made upon 3 groups of animals: Rats of Group I (Table I) were subjected to a "sham" adrenalectomy and allowed to drink tap water; those of Group II were adrenalectomized and allowed to drink 1% NaCl only; those of Group III were adrenalectomized and immediately started on a daily dose of 0.5 mg desoxycorticosterone acetate in oil subcutaneously. All rats were fed the stock diet for 10 days after adrenalectomy and then subjected to fasting. From the second day† of fasting until death the urinary nitrogen was determined daily. The survival period in days and the daily nitrogen excretion of these animals are shown in Table I. It will be seen that the salt-treated adrenalectomized rat is able to survive an average of 5.5 days of fasting. This approaches the survival period (6.3 days) of the adrenalectomized rats treated with desoxycorticosterone acetate. The sham adrenalectomized rats

TABLE I.

Nitrogen Excretion and Survival Time of Fasted Adrenalectomized Rats Allowed to Drink 1% NaCl as Contrasted with Sham Adrenalectomized and Hormone Treated Controls.

| | Urinary N mg per day<br>Day of fasting | | | | Fasting survival period, days |
|---|---|---|---|---|---|
| Group No. | 2nd | 3rd | 4th | 5th | |
| I. Sham adrenalectomized rats on tap water | 146 (8)†<br>(120-180)‡ | 132 (8)<br>(108-166) | 133 (8)<br>(106-170) | 133 (8)<br>(108-162) | 13<br>(10-17) |
| II. Adrenalectomized rats on 1% NaCl | 133 (9)<br>(116-154) | 107 (7)<br>(102-118) | 98 (6)<br>(94-102) | 97 (5)<br>(92-104) | 5.5<br>(3-10) |
| III. Adrenalectomized rats on DOCA* 0.5 mg daily | 125 (8)<br>(76-146) | 97 (7)<br>(88-112) | 91 (5)<br>(80-104) | 91 (5)<br>(82-106) | 6.3<br>(4-16) |

Each group was given the treatment indicated from the time of operation until death due to fasting. Fasting was started on 10th day postoperative.

*Desoxycorticosterone acetate in sesame oil.

†Number of animals in parentheses.

‡Range of values in parentheses.

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[1] Anderson, E., Herring, V., and Joseph M., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 488.

† The urinary nitrogen output was not determined on the first day of fasting since we did not have an arrangement for separating feces from urine. After the first day of fasting very few feces were passed.

survived an average of 13 days. On the second day of fasting the urinary nitrogen output of both groups of treated adrenalectomized rats (salt or desoxycorticosterone) was only slightly less than that of the sham adrenalectomized controls but after the second day of fasting their output was about 30% less than that of the controls.

In another set of experiments similar studies were made upon 4 groups of animals consisting of sham adrenalectomized rats and adrenalectomized rats given tap water, NaCl and desoxycorticosterone. In order to have all of the adrenalectomized rats in a comparable state of health at the beginning of the fast, adrenal cortical extract (Upjohn) was given for the 5 days after adrenalectomy during which time the animals were fed, and on the 6th postoperative day, which was the first day of the fast. A daily dose of 0.5 cc adrenal cortical extract (Upjohn) was given the first 4 days, and then increased to 1 cc for the next 2 days.

Beginning the first day of the fast the 4 groups of animals were given the following treatment: Group I, sham adrenalectomized

TABLE II.
Nitrogen Excretion and Survival Time of Fasted Adrenalectomized Rats Allowed to Drink Tap Water or 1% NaCl as Contrasted with Normal Controls and Hormone Treated Animals.

| | Fed—5 days postoperative | | Change in B.W. during fast, | Fluid intake during fast, | Urinary N mg per day Day of fasting | | | | | | Fasting survival period, |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group No. | Food intake, g/day | Water intake, cc/day | g/day | cc/day | 2nd | 3rd | 4th | 5th | 6th | 7th | days |
| I. Control sham adrenalectomized. Tap water during fasting | 17 | 34 | —8 | 14 | 144<br>(5)†<br>(134-158)‡ | 131<br>(5)<br>(127-142) | 128<br>(5)<br>(112-134) | 121<br>(5)<br>(110-132) | 111<br>(5)<br>(102-126) | 114<br>(5)<br>(100-124) | Fed on 8th day |
| II. Adrenalectomized.* Tap water during fasting | 6.4 | 20 | —7 | 9 | 78<br>(5)<br>(54-94) | 59<br>(4)<br>(50-58) | 40<br>(4)<br>(30 52) | 36<br>(1) | — | — | 4.8<br>(3-6) |
| III. Adrenalectomized* 1% NaCl during fasting | 8 | 22 | —6 | 18 | 134<br>(5)<br>(114-156) | 126<br>(5)<br>(116-132) | 122<br>(3)<br>(112-136) | 110<br>(3)<br>(104-116) | 93<br>(2)<br>(84-102) | 85<br>(2)<br>(76-94) | 6.4<br>(4-9) |
| IV. Adrenalectomized.* Tap water and 0.5 mg DOCA** | 6.6 | 17 | —7 | 8 | 140<br>(3)<br>(134-146) | 133<br>(3)<br>(124-140) | 122<br>(3)<br>(114-130) | 116<br>(3)<br>(112-120) | 98<br>(2)<br>(96-100) | 86<br>(1) | 7<br>(6-8) |

*The adrenalectomized rats were given adrenal cortical extract (Upjohn) 0.5 cc daily for 4 days postoperative; 1 cc on 5th day and 1 cc on 6th day or first day of fasting.

**Desoxycorticosterone acetate in sesame oil, daily during fast.

†Number of animals in parentheses.

‡Range of values in parentheses.

rats, had tap water to drink; Group II, adrenalectomized rats, had tap water to drink; Group III, adrenalectomized rats, were given 1% NaCl to drink; Group IV, adrenalectomized rats, were given tap water to drink and injected subcutaneously with 0.5 mg desoxycorticosterone acetate daily. The data of this study are shown in Table II. The untreated adrenalectomized rats were the least capable of enduring a fast. Their survival period averaged 4.8 days. The adrenalectomized rats which received NaCl or desoxycorticosterone survived an average of 6.4 and 7 days, respectively. The sham adrenalectomized rats were all alive on the 8th day of the fast when the experiment was terminated.

The untreated adrenalectomized rats showed a marked drop in urinary nitrogen when compared with the controls within 24 hours after stopping the cortical extract. The animals treated with NaCl and desoxycorticosterone showed a urinary nitrogen output which was practically the same as that of the controls through the fifth day of fasting.

In the early stages of acute adrenal insufficiency the output of urinary Na is increased, and that of the K is decreased. As the state of insufficiency advances the urinary Na is decreased due to a decreased blood flow through the kidney. In order to rule out a nitrogen retention due to diminished blood flow through the kidney in the case of the untreated adrenalectomized rat during the second and third days of fasting, the urinary excretions of Na and K were determined in conjunction with the nitrogen output. These data are shown in Table III. The animals of this group were treated in

TABLE III.
Excretion of N, Na and K in Fasted Adrenalectomized Rats.

| | Change B.W. g/day | | Water intake, cc/day | | Urinary output mg per day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2nd day fast | | | 3rd day fast | | |
| Group No. | Fed | Fasted | Fed | Fasted | N | Na | K | N | Na | K |
| I. Sham adrenalectomy, tap water to drink | | | | | | | | | | |
| W 5769 | +3 | —8 | 29 | 13 | 150 | 38 | 25 | 134 | 44 | 15 |
| W 5776 | +6 | —6 | 30 | 13 | 162 | 46 | 20 | 148 | 40 | 12 |
| II. Adrenalectomy, tap water to drink | | | | | | | | | | |
| W 5710 | —1 | —9 | 16 | 11 | 63 | 104 | 9 | 68 | 90 | 5 |
| W 5748 | +1 | —8 | 16 | 11 | 74 | 96 | 6 | 56 | 110 | 7 |

The animals were fed the first 5 days postoperatively and given tap water to drink. The adrenalectomized rats had 1 cc adrenal cortical hormone daily from time of operation through the first day of fasting.

a similar fashion as those of Groups I and II (Table II). The 2 adrenalectomized rats were given adrenal cortical extract, 1 cc daily for the 5 days postoperative during which time they were fed and on the sixth day postoperative which was the first day of the fast. During the second and third days of fasting, the urinary N, Na, and K were determined. It will be noted that the Na excretion is high for the adrenalectomized rats at the same time that the nitrogen excretion is low. It would seem from this that the low nitrogen excretion is not due to decreased renal blood flow but more probably to a diminished protein catabolism. From a comparison of the nitrogen excretion values of the 3 groups of adrenalectomized rats it is apparent that the rats treated with NaCl or desoxycorticosterone have a much greater protein breakdown during fasting than the untreated adrenalectomized rat, and that in fact, the rate of breakdown parallels that of normal rats for at least 5 days of fasting.

*Conclusion.* Fasted adrenalectomized rats treated with NaCl excrete nitrogen in amounts comparable with that of hormone-treated controls or that of normal animals, for at least 5 days of fasting. The survival time of such animals is also somewhat prolonged.

### 11983

## Study of the Serum Lipids in Sclerema Neonatorum.

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Although a number of investigations[1-6] have been made in sclerema neonatorum, no report has been found which deals with lipid analyses both at the time the disease was manifest and again when all evidence of the disorder had disappeared. Furthermore, since no studies of the blood lipids in this condition have been reported, it appeared that this approach to the problem might help to better understand the nature of this disturbance and to detect evidence of any metabolic abnormality which may be associated with this condition.

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* Harold Rypins, Research Assistant.

[1] Harrison, G. A., and McNee, J. W., *Arch. Dis. Child.*, 1926, **1**, 63.

[2] Harrison, G. A., *Arch. Dis. Child.*, 1926, **1**, 123.

[3] Channon, H. C., and Harrison, G. A., *Biochem. J.*, 1926, **20**, 84.

[4] Kohnstam, G. L. S., and Herbert, F. K., *Arch. Dis. Child.*, 1927, **2**, 349.

[5] Bernheim-Karrer, J., *Z. f. Kindh.*, 1933, **55**, 695.

[6] McIntosh, J. E., Waugh, T. R., and Ross, S. G., *Am. J. Dis. Child.*, 1938, **55**, 112.

A 3½-month-old, breast-fed female infant was referred to the University of Minnesota Hospital because of failure to gain weight and the persistence of many hard patches of subcutaneous tissue. The skin covering these firm areas had a slightly bluish tinge while that over the rest of the body was dry, wrinkled and thin. Our clinical observation that these abnormal patches of bacon-like consistency persisted in spite of a marked scarcity of normal subcutaneous adipose tissue in the uninvolved areas of the body suggested that this pathologic tissue was metabolically altered as well as physically and chemically. Shortly after admission to the hospital a biopsy was obtained from one of the abnormal areas in the left thigh. The histologic appearance of this tissue was the same as described by various workers.[1, 4, 5, 6] Chemical analysis of this tissue was made by the same technic as used by Hansen and McQuarrie[7] in the study of a child with universal lipodystrophy. The tissue of the opposite thigh which previously had been involved was examined in a similar manner 8 months later when all evidence of the disease had vanished. The results of the chemical studies on the tissues obtained early were essentially the same as found by previous workers, the iodine number of the tissue fat being 42 at this time. Subsequently, when all signs of the disease had disappeared, the iodine number had risen to 57.

At the time of admission of this patient to the hospital, routine examination of the blood disclosed that the serum had a milky appearance and contained 285 mg cholesterol per 100 cc. Due to the development of an upper respiratory infection complicated by suppurative otitis media, blood studies were delayed about 6 weeks, however, at this time the characteristic abnormal, hard patches of subcutaneous tissue had changed very little. In addition to the preliminary study on a fasting sample, blood was obtained before and 4 hours following the administration of 4 ounces of 35% cream. The total fatty acids of the serum were determined by the method of Wilson and Hansen[8] and the acetone insoluble (phospholipid) and the acetone soluble (cholesterol ester-neutral fat)

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[7] Hansen, A. E., and McQuarrie, I., PROC. SOC. EXP. BIOL. AND MED., 1940, 44, 611.

[8] Wilson, W. R., and Hansen, A. E., *J. Biol. Chem.*, 1936, **112**, 457.

TABLE I.
Values of Various Fractions of Serum Lipids in an Infant with Sclerema Neonatorum During the Course of the Disease and Following Recovery.

| Condition | | Fasting | Fasting | Fat* meal | Fasting | Fat meal | Fasting | Fat meal | Normal† |
|---|---|---|---|---|---|---|---|---|---|
| Date | | 2-9 | 3-2 | 3-2 | 3-25 | 3-25 | 8-8 | 8-8 | |
| Unsaponifiable | mg% | 364.9 | 278.2 | 298.3 | 223.6 | 246.7 | 169.9 | 200.2 | |
| Saponifiable | mg% | 901.4 | 693.6 | 1335.7 | 395.6 | 982.5 | 290.8 | 593.2 | 350.1 |
| (T.F.A.) | I.N. | 80.0 | 88.1 | 58.6 | 90.2 | 69.5 | 83.4 | 67.4 | 107.4 |
| Acetone insoluble | mg% | 180.0 | 160.7 | 184.3 | 118.4 | 124.2 | 88.5 | 123.4 | 107.7 |
| (P.L.F.A.) | I.N. | 99.6 | 113.5 | 92.8 | 93.5 | 77.1 | 90.4 | 93.2 | 112.8 |
| Acetone soluble | mg% | 685.5 | 505.0 | 1157.6 | 270.5 | 793.2 | 197.4 | 476.9 | |
| (C.E.-N.F.F.A.) | I.N. | 76.7 | 78.2 | 50.6 | 75.5 | 59.2 | 82.0 | 62.8 | |
| Cholesterol | | | | | | | | | |
| Total | mg% | | 275.0 | | 174.0 | 179.8 | 137.2 | 153.35 | |
| Combined | mg% | | | | 91.6 | 141.6 | 83.3 | 93.97 | |
| Free | mg% | | | | 82.5 | 38.1 | 53.9 | 59.38 | |
| | % of total | | | | 47.4 | 21.2 | 39.3 | 38.72 | |

*4 hr after 4 oz 35% cream.
†Average of 18 determinations in 14 infants and children.[12]

fractions by the same method used by Hansen.[9] The cholesterol and cholesterol esters were measured by the method of Bloor.[10, 11]

The results are presented in Table I.

*Discussion.* It is quite apparent that a lipemia was present in this subject. The total fatty acids of the serum at the beginning of this study (2/9/40) were found to be almost 3 times the average value for normal infants.[12] Part of this increase was due to an elevation in the phospholipid (acetone insoluble) fraction. Although the cholesterol esters were not determined at this time the abnormally high value for the unsaponifiable fraction would indicate that these fatty acids were increased. However, the greatest increase was in the neutral fat fatty acids. If we assume that the cholesterol ester fatty acids tend to be highly unsaturated and remain rather constant in composition[13] the neutral fat fatty acids probably were very saturated and had a degree of unsaturation somewhat similar to that found in the tissue, which had an iodine number of 42.

The most striking finding in these studies was the tendency for all fractions of the serum lipids gradually to drop to the normal range as improvement in the condition occurred. The degree of unsaturation of the phospholipid fatty acids varied considerably. On the other hand the iodine number of the acetone-soluble fraction of the serum lipids steadily increased. In the final specimen, obtained when the child was clinically well (8/7/40), the calculated iodine number of the neutral fat fatty acids of the serum apparently had increased and was probably in the same range as that of the neutral fat fatty acids in the tissue. The iodine number of the neutral fat in the tissues was 57 at this time.

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[9] Hansen, A. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 376

[10] Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

[11] Bloor, W. R., and Knudson, A., *J. Biol. Chem.*, 1916, **27**, 107.

[12] Hansen, A. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 205.

[13] Schaible, P. J., *J. Biol. Chem.*, 1932, **95**, 79.

From the absorption studies it is quite apparent that fat was readily absorbed into the blood stream in this patient. In fact the increase in the level of the blood fat seemed rather excessive[14] when first studied as compared with the rise in blood fat that occurred later. It was impossible to run a complete fat absorption curve; therefore, one cannot draw any definite conclusions. Fat balance studies on this patient showed normal absorption from the gastrointestinal tract and normal retention.

It is obvious that none of the more common causes of lipemia such as diabetes mellitus, lipoid nephrosis and glycogen storage disease was present in our case. There is no evidence from the work of other observers that the liver is abnormal in sclerema neonatorum; therefore, the lipemia cannot be explained on the basis of associated hepatic disease. It is well known that various types of xanthomatoses are associated with an increase in the blood fats, however, it is entirely clear that we are not dealing with such a phenomenon in this condition. Two factors which must be given serious consideration in explaining the findings in our case are those related to the intermediary metabolism of fat. First, lipemia may occur when there is interference with the deposition of fat in the normal storage compartments, and second, when the mobilization of fat from the tissues exceeds its utilization. These features may be particularly significant when we realize that distinct changes are present in sclerematous tissue. As previously stated, our histologic and chemical studies confirm the findings of others, namely, that there is a chronic, inflammatory reaction similar to the type produced by a foreign body and that the fatty acids tend to be more saturated and to consist of shorter chains (probably a replacement of some oleic acid with palmitic acid).

It has not been mentioned previously as to whether or not a systemic effect is produced or associated with these changes. The finding that a lipemia occurred which disappeared with improvement in the clinical condition suggests that a metabolic abnormality existed in our subject. This seems to confirm our clinical impression that the fat in this pathologic tissue is altered metabolically. So little is known concerning the hormonal and neurogenic mechanisms, as well as the local factors, such as enzymes, which may play a rôle in fat metabolism that only brief mention may be made here of these phases of the problem. We have no evidence that either thyroid or pituitary dysfunction was present in our subject. One cannot rule out the fact that certain neurogenic influences may be involved in this disorder, but more work is necessary to fully understand the significance of this phase of lipid metabolism. McIntosh, Waugh and Ross[6] could demonstrate no evidence of lipase activity in the tissues of their subject, however, results of control studies were not given. From a consideration of the various factors concerned with lipid metabolism it would seem that the local changes in the tissue may interfere with the normal deposition or mobilization of fat sufficient to produce the lipemia. As recovery occurs this abnormality disappears.

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[14] Wilson, W. R., and Hanner, J. P., *J. Biol. Chem.*, 1934, **106**, 323.

*Conclusions.* Analyses of the subcutaneous tissues in a 3½-month-old, breast-fed female infant with sclerema neonatorum revealed the usual histologic and chemical alterations: chronic inflammatory reaction and presence of excess saturated fat. When the condition was improved repeat analyses revealed normal adipose tissue. During the course of the disease studies of the blood lipids were made. Early in the disease an increase in all the fractions of the blood lipids as well as a decrease in the iodine number, especially of the neutral fat, was found. When all signs of the disease had disappeared the blood lipids were normal. The lipemia is briefly discussed in the light of the alterations of the fat tissues, and it is suggested that a disturbance in fat deposition or in fat transport from the pathologic areas may be present in this disease. The clinical observation gives support to the idea that this tissue was metabolically altered.

### 11984

## Use of Colchicine in Detecting Hormonal Effects on Vaginal Epithelium of Menstruating and Castrate Women.*

Ephraim Shorr and Eugene J. Cohen.

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The interesting observation that colchicine accumulates dividing cells in metaphase by preventing a completion of mitotic division has been made use of in the study of the action of estrogens,[1] androgens,[2] and gonadotropins[3] in animals. The great increase in the number of mitotic figures obtained with the colchicine technic over the small number seen when the hormones are used alone, has served to throw light on several previously obscure aspects of the action of these hormones.

The use of the colchicine technic in relation to hormonal action has hitherto been confined to animals, presumably because doses comparable to those effective in animals would exceed the limit of safety for this drug in man. However, it seemed likely that this technic could be safely applied to man were the alkaloid brought, in adequate concentrations, in direct contact with accessible structures like the vaginal epithelium and skin, and the total amount kept within recognizably safe limits. This report deals with experiments designed to explore the usefulness of the colchicine technic in revealing the effects of various hormones on the vaginal mucosa of menopausal castrates and menstruating women.

*Methods.* Colchicine was incorporated into gelatin pessaries for insertion into the vaginal vault. Vaginal biopsies were taken 17-24 hours after insertion of pessaries containing 1-3 mg of the alkaloid. A number of control biopsies were taken just prior to the insertion of the pessary. The subjects were 4 menopausal castrates and 6 menstruating women. Vaginal smears were taken daily to ascertain the effects of the hormones used with the castrate group.

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* Aided by grants from the Council on Pharmacy and Chemistry of the American Medical Association, and the Wyeth Endocrine Fund.

The estradiol benzoate (Progynon B) and progesterone (Proluton) were kindly furnished by Dr. Erwin Schwenk of the Schering Corporation.

[1] Allen, E., Smith, G. M., and Gardner, W. U., *Am. J. Anatomy*, 1937, **61**, 321.

[2] Martins, T., *Compt. rend. Soc. de Biol.*, 1937, **126**, 131; Fleischmann, W., and Kann, S., *Adak. Anz. Akad. d. Wissensch. Wien*, 1937, No. 21; *Biochem. Z.*, 1938, **296**, 373.

[3] Allen, E., Smith, G. M., and Gardner, W. U., *Anat. Rec.* (Suppl. 3) 1937, **67**, 3.

and to guide the selection of the appropriate time for biopsy in the menstruating group. The smears were stained by the method recently developed in this laboratory.[4] The biopsies, 69 in number, were fixed either in Carnoy's or Flemming's solution. After the former fixative they were stained by a modified Masson technic, after the latter, by Safranin and Fast Green F.C.F.

*Experimental Procedures.* (1) *The menopausal castrate group:* Estradiol benzoate was given intramuscularly in ascending doses until a dose was reached which produced a fully cornified follicular smear. This amount of estrogen was then given concomitantly with progesterone in amounts of from 25-50 mg daily for a period of 2-3 weeks (Table I). It has been previously shown[5] that under this regime the cornified vaginal smears induced by estrogens assumed the characteristics of the vaginal secretion of the second half of the menstrual cycle, and that the vaginal epithelium was transformed from the induced mid-menstrual type to that found in premenstruum or, with the larger doses of progesterone, in pregnancy. Vaginal biopsies following the insertion of colchicine pessaries were taken at various stages of treatment.

(2) *The Menstruating Group:* No hormonal therapy was given, but vaginal biopsies after colchicine pessaries were taken when

TABLE I.
A. Menopausal Castrate Group.
Schedule of Hormone Treatment.

| Case | Estradiol benzoate estrous unit and maintenance dose R.U. per day | Progesterone, mg (No. days) |
|---|---|---|
| 1 | 5,000 | 25(6), 35(9) |
| 2 | 4,000 | 25(8), 35(3), 50(2) |
| 3 | 10,000 | — |
| 4 | 3,000 | 25(8), 35(9) |

B. Menstruating Women.
Day of Biopsy in Relation to Menstrual Cycle.

| Case | Menstrual cycle, days | Day of biopsy |
|---|---|---|
| 5 | 28 | 14, 27 |
| 6 | 26-30 | 27 |
| 7 | 27-28 | 11, 17, 24 |
| 8 | 23-26 | 11, 25 |
| 9 | 23-26 | 25 |
| 10 | 28 | 13 |

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[4] Shorr, E., *Science*, 1940, **91**, 321, 579.

[5] Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 501.

vaginal smears showed (a) maximal follicular activity and (b) during the last week of the premenstruum.

*Results.* The findings are presented as qualitative rather than quantitative evidence of the action of colchicine on the vaginal mucosa under these experimental conditions, since some leakage from the vagina could not be prevented, and there were differences in the time after insertion of the pessaries at which the biopsies were taken. No symptoms attributable to colchicine were encountered.

The changes in the vaginal smears under the hormonal regime described, were in agreement with those previously reported from this laboratory,[5] the cornified smear induced by estrogens assuming the appearance characteristic of the premenstrual phase of the normal menstrual cycle[6] when progesterone was added. The structure of the vaginal epithelium was altered by the combined progesterone-estrogen treatment in the manner previously described,[5] the extent of the increased proliferation varying directly with the amount of progesterone used.

The chief point of interest lay in the increased number of mitoses which accumulated as a result of the action of colchicine. Under these experimental conditions, vaginal biopsies taken without colchicine show only an occasional mitotic figure in spite of the obvious proliferation. After colchicine, except when leakage or expulsion of the pessary was reported by the patient, a significant increase in the number of mitotic figures occurred. In the *menopausal group*, an increase in the number of arrested mitotic figures was seen following estradiol benzoate alone and in combination with progesterone. In the *menstruating group,* mitotic figures indicative of active proliferation were seen during both the follicular and premenstrual phases.

The mitotic figures were largely confined to the lower layers of the basalis adjacent to the stratum germinativum. Their distribution was patchy, indicating that proliferation was not taking place uniformly throughout the whole epithelium. Except for a rare figure in anaphase, the arrested mitoses were in pro- or metaphase; and there was a high percentage of atypical mitotic figures. Among the latter could be distinguished cells with densely packed chromatin which were considered to represent an atypical prophase in process of degeneration. Cells in atypical metaphase, with the chromosomes grouped to one side of the spindle, were frequent. Many of the cells in arrested division were much larger than the resting cells,

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[6] Papanicolaou, G. N., *Am. J. Anat.* (Suppl.), 1933, **52**, 519.

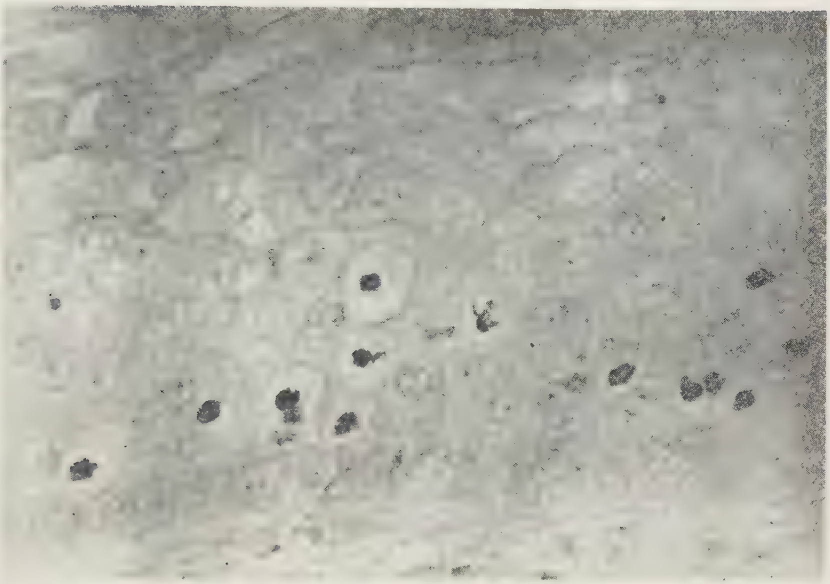

FIG. 1. (Case 7—normal cycle.)
Vaginal epithelium during follicular phase. (11th day.)

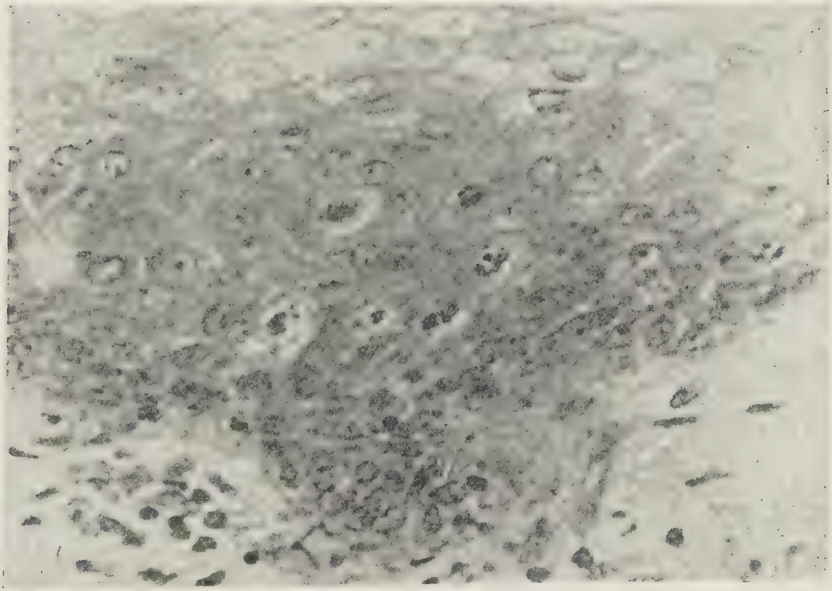

FIG. 2. (Case 7—normal cycle.)
Vaginal epithelium during premenstrual phase. (24th day.)

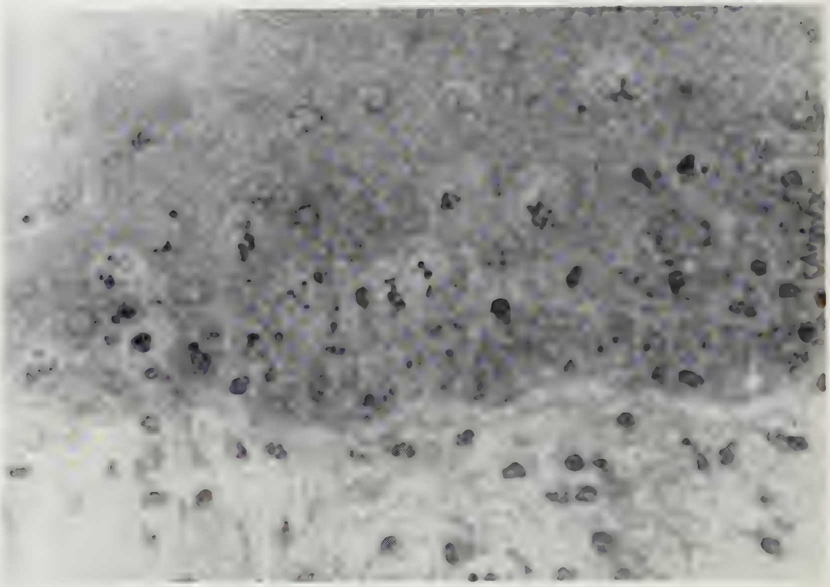

FIG. 3. (Case 2—menopausal castrate.)
Vaginal epithelium during induced follicular phase.

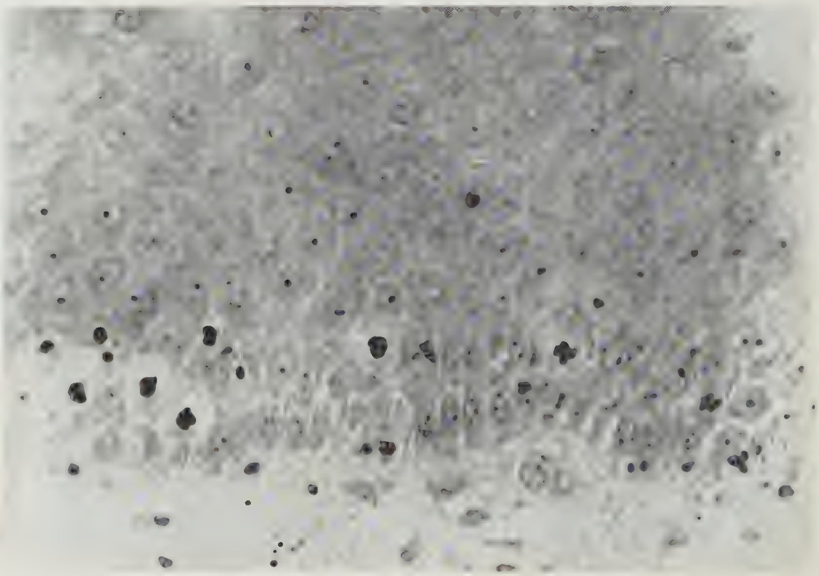

FIG. 4. (Case 2—menopausal castrate.)
Vaginal epithelium during estrogen-progesterone administration.

many were vacuolated, and the frequency with which they were devoid of cytoplasm and chromosomes suggested their fragility.

*Discussion.* The applicability of the colchicine technic to man makes available an additional method for analyzing hormonal effects on structures such as the vagina and skin which are accessible to topical application of the alkaloid. It may also be possible to introduce colchicine in a suitable medium into the uterine cavity prior to endometrial biopsies. What additional information will be derived from the use of this technic can only be determined by further experiments.

The technic was employed in the present study to aid in the analysis of the hormonal factors responsible for the structure of the vaginal epithelium during the menstrual cycle and thereby responsible for the character of the desquamated vaginal secretion. The results reinforce the conclusions drawn from previous experiments of the same character in which colchicine was not employed.

The effects of estrogens in stimulating the growth of the vaginal mucosa and producing a cornified vaginal secretion[7, 8] are now generally accepted, and the accumulated mitoses, after estrogens in the menopause and during the follicular phase of the normal cycle, reflect this proliferative influence. The conclusions were drawn from our previous study[5] that the estrogen-progesterone combination which controls the second half of the menstrual cycle, has two effects: (a) to modify the estrogen effect on the vaginal epithelial cell so that it fails to develop to full cornification, and (b) to enhance the proliferative effects of estrogens in a manner analogous to its action on endometrial growth. The abundant mitoses seen in premenstruum in normal women, and in castrates when this combination of hormones is given, support the conclusion that active proliferation is taking place.

*Conclusions.* Colchicine, by topical application in vaginal pessaries, permits the detection of the growth stimulating effects of estrogens, and progesterone in combination with estrogens, on the vaginal epithelium of menopausal castrates. In menstruating women, this technic demonstrates clearly the proliferative effect of these hormones of endogenous origin during both the follicular and premenstrual phases of the cycle.

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[7] Davis, M. E., and Hartman, C. G., *J. A. M. A.*, 1935, **104**, 279.

[8] Papanicolaou, G., and Shorr, E., *Am. J. Obs. and Gyn.*, 1936, **31**, 3.

### 11985

## Androgenic Action of Desoxycorticosterone Acetate?

KARL E. PASCHKIS.* (Introduced by Hobart A. Reimann.)
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It is generally assumed that under certain circumstances the adrenals exert an androgenic action. Clinical evidence may be seen in the high excretion of androgens in women with tumors of the adrenal gland as well as in the fact that both excretion of androgens and clinical symptoms of virilism regress after removal of the adrenal tumor. In the young rat and mouse, castration is not followed by complete atrophy of the prostate and seminal vesicles[1, 2] unless the adrenals are also removed.[4, 3] The X zone of the mouse adrenal is considered to be androgenic[5] but this interpretation has been doubted and the X zone was thought to represent a "reserve zone of the adrenal".[6]

Reichstein has isolated andrenosterol from the adrenal cortex and has proved its androgenic action.[7] This, however, is probably not the only androgenic steroid compound of the adrenal cortex. Progesterone, one of the sterols found in the adrenal cortex, is androgenic.[8] It was, therefore, planned to examine sterols of the cortex for their androgenic activity. In this paper we report on desoxycorticosterone acetate. Other sterols are to be studied if and when sufficient quantities become available to us. Desoxycorticosterone has been studied previously with conflicting results. Hooker and Collins[9] have found this compound to be active, whereas Greene and Burrill[10] failed to find any androgenic activity.

Our interest in the possible androgenic action of various cortical steroid compounds was also stimulated by the question whether cases of virilism, in which no disturbance of salt and water metabolism or of carbohydrate metabolism could be detected, might be explained on the basis of "dissociated hyperfunction" of the adrenal cortex (unpublished observations).

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* J. Ewing Mears Fellow.

[1] Price, D., *Am. J. Anat.*, 1936, **60**, 79.

[2] Howard, E., *Am. J. Physiol.*, 1937, **119**, 339.

[3] Burrill, M. W., and Greene, R. R., PROC. SOC. EXP. BIOL. AND MED., **1939, 40**, 327.

[4] Burrill, M. W., and Greene, R. R., *Endocrinology*, 1940, **26**, 645.

[5] Howard, E., *Am. J. Anat.*, 1939, **65**, 105.

[6] Gersh, T., and Grollman, A., *Anat. Rec.*, 1939, **75**, 131.

[7] Reichstein, T., *Helv. Chim. Acta.*, 1936, **19**, 223.

[8] Greene, R. R., Burrill, M. W., and Ivy, A. C., *Endocrinology*, **1939, 24, 353.**

[9] Hooker, Chas. W., and Collins, V. J., *Endocrinology*, 1940, **26**, 269.

[10] Greene, R. R., and Burrill, M. W., PROC. SOC. EXP. BIOL. AND MED., **1940, 43**, 382.

*Methods and Results.* For assay of androgenic activity, two methods were used: (a) stimulation of mitosis in the prostate and seminal vesicle of castrate rats as described by Fleischmann,[11] and (b) the comb growth of the immature chick (Frank,[12] modified by Rakoff[13]).

Twenty adult male rats weighing between 200 and 250 g were castrated. Adult instead of immature rats as employed by Fleischmann were used in order to exclude any possible influence of the animals' own adrenals (see above). Three and one-half weeks were allowed for complete atrophy of the accessories (prostate, seminal vesicles). Desoxycorticosterone acetate* was then injected subcutaneously in two divided doses (first half dose at 2 p. m., second half dose at 8 a. m. on the following morning). Two tenths mg colchicine were injected simultaneously with the second half dose, but at a different site. Eight hours after the injection of the colchicine, *i. e.,* 26 hours after the first injection of hormone, the animals were killed. The prostate and seminal vesicle were fixed in Bouin's solution, imbedded in paraffin, sectioned and stained with hematoxylin and eosin. From each animal a specimen from the small intestine was treated in the same way in order to serve as a control for the effectiveness of the colchicine to arrest mitoses.

The animals were divided into 4 groups, one group serving as control and receiving colchicine only, the 3 others receiving desoxycorticosterone acetate in total doses of 0.6, 1.0, 5.0 mg, respectively.

There was no stimulation of mitosis in the prostate or seminal vesicle at either of the 3 dosage levels employed. Mitoses were very plentiful in the intestinal epithelium, thus indicating normal response of the animals to colchicine.

*Comb Growth Promoting Potency.* It is well known that some androgens, such as testosterone and its esters, are relatively more potent in repair of the rodent's accessories; whereas others, such as androsterone, have a relatively higher comb growth promoting potency. Since the experiments on the castrated rat had revealed no androgenic potency, the following experiments on one-day-old chicks were carried out. Thirty chicks were used. The hormones were injected into the comb. Six chicks received 0.025 mg desoxycorticosterone daily for 6 days, a total of 0.15 mg; 6 chicks received 0.125 mg daily for 6 days, a total of 0.75 mg. Six animals received no treatment (untreated controls); 5 were treated with androsterone, receiving 6 daily doses of 0.005 mg, a total of 0.03 mg; 6 received 6 daily doses of 0.0005 mg of androsterone, a total of 0.003 mg (androsterone controls). Whereas treatment with androsterone produced an increase of comb growth of 20 and 32% respectively with the dosage levels employed, there was no increase in comb growth with desoxycorticosterone acetate; rather the desoxycorticosterone acetate seemed possibly to impair comb growth. The comb weight in the desoxycorticosterone-treated animals was 9 and 12% lower than that of the untreated controls. These figures are probably not significant.

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[11] Fleischmann, W., *Endocrinology,* 1939, **25**, 798.

[12] Frank, R. T., Klempner, E., and Hollander, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 853.

[13] Rakoff, A., unpublished.

* The author is indebted to Dr. E. Schwenk of the Schering Corporation for generous supply of desoxycorticosterone acetate (Cortate).

No androgenic activity of desoxycorticosterone acetate was found either in the castrate rat or in the chick. These findings are in agreement with the results of Green and Burrill.[10] These authors gauged the androgenic activity by maintenance of secretion of the ventral prostate of the castrate rat, as judged by histologic appearance. In our experiments stimulation of mitosis in the castrate rats' accessories was used as indicator of androgenic activity. This is probably a more sensitive test. The question of specificity of this test need not be discussed since the results were negative. Howard[14] has shown that even large doses of desoxycorticosterone fail to produce atrophy of the X zone of the adrenal gland of the mouse. Atrophy of the X zone is the typical reaction of the adrenal gland of the mouse to androgens. Hooker and Collins[9] have based their statement of androgenic activity of desoxycorticosterone acetate on increase of seminal vesicle weight in 2 desoxycorticosterone-treated rats over that of one control. In addition they report on 6 capons treated with varying dosage levels. In our experiments desoxycorticosterone acetate failed to stimulate comb growth in 12 chicks.

*Summary.* Desoxycorticosterone is not androgenic.

The author gratefully acknowledges the technical assistance of Miss Margaret R. Kelleher.

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[14] Howard, E., *Anat. Rec.*, 1940, **77**, 181.

### 11986 P

## A Color Reaction Given by Some $\alpha$-Amino Acids.

ALTON C. KURTZ. (Introduced by D. W. Wilson.)

*From the Department of Physiological Chemistry, Medical School, University of Pennsylvania, Philadelphia.*

A color reaction given by certain $\alpha$-amino acids was found during a study of the p-nitrobenzoylation of some of them. When a few milligrams of a reactive amino acid are dissolved in 2.0 cc of 5% sodium hydroxide in a test tube, cooled, and vigorously shaken for half a minute with 2.0 cc of a saturated solution of p-nitrobenzoyl chloride in carbon tetrachloride, no color develops. However, if 5 or 10 cc of n-butyl alcohol are now added and the tube is thoroughly agitated for a few seconds, a violet color appears in the butyl alcohol phase. The color persists for a few minutes and then gradually fades, becoming reddish and then colorless or pale yellow.

The violet color was given by *dl*-alanine, *dl*-valine, *dl*-norvaline, leucine, *dl*-norleucine, *dl*-phenylalanine, tyrosine, methionine, cystine, tryptophane, histidine, *dl*-lysine, *dl*-ornithine, $\alpha, \gamma$-diaminobutyric acid, arginine, and *dl*-citrulline. The test was negative on aspartic acid, glutamic acid, and hydroxyproline, and very faint on proline. It is presumed that the latter compound was not strictly pure. Glycine and *dl*-threonine gave dull orange colors, sarcosine a yellow, and glycine ethyl ester a pinkish brown. Negative results were obtained with every other substance tested, including the following: ammonia, $\beta$-alanine, urea, creatinine, adenine, uric acid, glycyl-*l*-leucine, ethyl allophanate, taurine, choline, brucine, $\alpha$-brom-$\epsilon$-benzoylaminocaproic acid, succinamide, m-aminobenzoic acid, and aniline.

When the volumes of reagents were those indicated above, the least amount of leucine which could be detected lay between 20 and 50 $\gamma$. The test was completely negative with the smaller quantity, but gave a good pink with the larger. The color produced was matched approximately by aqueous potassium permanganate.

Three different samples of p-nitrobenzoyl chloride gave similar results. One of the 3 samples was thrice recrystallized from carbon tetrachloride specially purified by successive thorough washings with mercury, concentrated sulfuric acid, sodium hydroxide solution, and water, followed by drying and distillation.[1] The recrystallized p-nitrobenzoyl chloride gave the color in undiminished intensity. The reagent may be dissolved in ether instead of in carbon tetrachloride with equally good color production when the solution is fresh, but after standing 2 weeks at room temperature the ethereal solution is no longer effective. Benzoyl chloride, m-nitrobenzoyl chloride, 3,5-dinitrobenzoyl chloride, p-brombenzoyl chloride, m-chlorbenzoyl chloride, and benzenesulfonyl chloride could not be substituted for p-nitrobenzoyl chloride. Other alcohols may be used in place of n-butyl alcohol. Color was produced with methanol (synthetic), ethanol (two different samples), isopropanol (distilled over lime), and isoamyl alcohol, but not by ether, ethyl acetate, acetone, or phenol.

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[1] Weissberger, A., and Proskauer, E., *Organic Solvents,* Oxford, 1935, p. 156.

The mechanism of the color production is not obvious. Isolated p-nitrobenzoyl leucine gave a positive test only when additional p-nitrobenzoyl chloride was used. If the butyl alcohol is added without agitation the violet color is produced at the junction of the aqueous and alcoholic layers and diffuses up into the latter where it eventually fades. The violet butyl alcohol solution becomes colorless on the addition of acetic acid, but the color is restored if excess sodium hydroxide is again added. When the acid solution is allowed to stand for some minutes restoration of the color by alkali is not possible. Aldehydes such as benzaldehyde and formaldehyde partly or completely inhibit the reaction, depending upon the conditions of the reaction and the amounts added. On dilution of the alcohol phase by nitrobenzene the color changes to blue; dilution by carbon tetrachloride causes the color to become red. There appears to be no analogy between the reaction described here and that given by phenylalanine according to Kapeller-Adler,[2] since in the present instance hydroxylamine completely inhibits color production. On the other hand, the reaction of alkaline alcoholic solutions of o- and m-dinitrobenzenes on fructose[3] gives colors which are similar in general behavior to those given in the present instance, particularly with respect to the changes in hue induced by dilution with suitable solvents.

*Summary.* A color reaction characteristic of many $\alpha$-amino acids has been described.

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[2] Kapeller-Adler, R., *Biochem. Z.*, 1932, **252**, 185.

[3] Joachimoglu, G., *Biochem. Z.*, 1915, **70**, 93.

## 11987

# Requirement of Potassium by the Chick.*

Ben-Ami Ben Dor. (Introduced by H. J. Almquist.)

*From the Division of Poultry Husbandry, College of Agriculture, University of California, Berkeley.*

In the search for a new chick growth factor present in rice bran and wheat bran, it was found that the beneficial effect of adding these substances to a rice-fish-meal basal diet was due, in part, to their high potassium content. Since no data could be traced in the literature on the potassium requirement of poultry, it seemed desirable to report certain results obtained in these studies. The capacity of rubidium to replace potassium was also investigated.

*Experimental.* Single Comb White Leghorn chicks procured from the University flock were reared on the normal stock ration until placed in experimental groups. They were kept in electrically heated brooders provided with wire screen floors. Ordinary tap water was supplied throughout the experiments.

The basal diet contained per 100 g, rice, polished, 71.0 g; fishmeal, water-washed and ether-extracted, 20.0 g; soybean oil, 3.0 g; vitamins A and D carrying oil, 1.0 g; hexane extract of alfalfa equivalent to 2.0 g of alfalfa. 2.2 g of minerals were added as a salt mixture containing sodium chloride, 46.0, calcium carbonate, 46.0, magnesium sulfate, 2.5, manganese sulfate, 2.4, ferrous sulfate, 1.25, aluminum sulfate, 1.25, zinc sulfate, 0.23, copper sulfate, 0.20, potassium iodide, 0.03, and cobalt acetate, 0.02 parts. This diet was supplemented with synthetic vitamins as follows: riboflavin, 1.0 mg; thiamin, 1.0 mg; nicotinic acid, 0.5 mg. 6.0 g of an extract of wheat bran from which the potassium was removed supplied pyridoxin and factor U.

The wheat bran extract was prepared by extracting 5 times with warm (50°C) 60-70% methanol (by volume). The combined extracts were concentrated under reduced pressure. Each pound of the final extract was derived from 6 pounds of bran. The chick antidermatitis vitamin (pantothenic acid) was supplied as 3.0 g of a concentrate prepared from whey from which the potassium was removed. It was prepared from the wash liquor remaining after the removal of lactose from whey, as described by Lepkovsky, Taylor, Jukes and Almquist.[1] The wheat bran extract and the chick antidermatitis vitamin concentrate were treated for the removal of potassium by the procedure of Miller.[2] Factor U was prepared by eluting with barium hydroxide a fullers' earth adsorbate prepared from the wheat bran extract. The charcoal eluate of the milk sugar concentrate was prepared by the method of Woolley, Waisman, Mickelsen and Elvehjem.[3]

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* The author wishes to acknowledge gratefully the help and advice of Drs. S. Lepkovsky, H. J. Almquist, and L. W. Taylor.

Synthetic vitamins used were kindly provided by Merck and Co., Inc.

Potassium and rubidium were added in the form of the chloride. The total potassium content of basal diets was determined by analysis.

*Effect of Potassium on Growth and Mortality of Chicks.* The results of one of the tests on the ability of chicks to grow and survive on different levels of potassium are given in Table I.

It is evident that under the conditions studied, more than 0.13% of dietary potassium is essential for good growth and survival.

*Replacement of Potassium by Rubidium.* The ability of rubidium to replace potassium was determined with the same basal diet with the following modifications. Wheat bran extract was eliminated and pyridoxin was supplied as 0.3 mg synthetic pyridoxin. Factor

TABLE I.
Weight and Mortality of Chicks 35 Days on Diets Supplemented with Different Levels of Potassium.*

| Total potassium in diet, % | Avg wt, g | Mortality, % |
|---|---|---|
| 0.076 | 139 | 71.5 |
| 0.130 | 272 | 43.0 |
| 0.230 | 280 | 14.5 |
| 0.400 | 286 | 0.0 |

*Each group began with 14 10-day-old chicks whose average weight was 62 g.

TABLE II.
Effect of Rubidium on Potassium Deficiency.

| Potassium and rubidium in diet % | Chicks per group | On 15th day on test diet* |  |  | On 28th day on test diets, No. chicks dead |
|---|---|---|---|---|---|
|  |  | No. chicks dead | Avg wt in g | Standard error |  |
| .072 K | 10 | 1 | 48 | 1.8 | 5 |
| .072 K + .072 Rb | 10 | 0 | 52 | 1.4 | 4 |
| .072 K + .28 Rb | 10 | 0 | 55 | 2.8 | 5 |
| .172 K | 14 | 0 | 73 | 4.5 | 0 |

*The average weight of the chicks on the first day on the test diets was 35 g. They were placed on the experimental diets at the time of hatching.

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[1] Lepkovsky, S., Taylor, L. W., Jukes, T. H., and Almquist, H. J., *Hilgardia*, 1938, **11**, 559.

[2] Miller, H. G., *J. B. C.*, 1923, **55**, 61.

[3] Woolley, D. W., Waisman, H. A., Mickelsen, O., and Elvehjem, C. A., *J. B. C.*, 1938, **125**, 715.

U was supplied as 0.7 g of a fullers' earth eluate of the wheat bran extract. Pantothenic acid was supplied as 0.1 g of a charcoal eluate of the milk sugar concentrate. The results are shown in Table II. Rubidium exerted a slight and only temporary alleviating effect on potassium deficiency.

*Discussion.* A comparison of the potassium requirement of the rat, as reported in the literature, and that of the chick, as reported in this paper, indicates a greater sensitivity on the part of the latter to potassium deficiency. When rats were fed 0.037% potassium[2] and 0.033% potassium[4] they survived at least 112 days and 220 days respectively. The chicks, on the other hand, were all dead within 40 days when fed 0.076% potassium. At the same time chicks receiving adequate levels of potassium grew fairly well and were in good condition. Growth, however, was below that obtainable on our stock diet.

*Summary.* 1. At least 0.17 g of potassium per 100 g of diet was found necessary to secure approximately maximal growth of chicks under the conditions of these experiments. 2. More than 0.13 g of potassium per 100 g of diet was necessary to prevent heavy mortality. 3. Rubidium had a slight and only temporary alleviating effect on potassium deficiency.

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### 11988

## Formation of Pressor Amines in the Kidney.

R. J. BING AND M. B. ZUCKER. (Introduced by M. I. Gregersen.)

*From the Department of Physiology, College of Physicians and Surgeons, Columbia University.*

Holtz[1, 2] found that renal cortical extracts of different species were able to transform *l*-histidine, *l*-tyrosine and *l*-dopa (*l*-dihydroxyphenylalanine) into their corresponding amines. The presence of the amines was demonstrated by their effect on the blood pressure of a test cat. The action of the enzymes responsible for this decarboxylation could be demonstrated only under anaerobic conditions. Bing[3] showed that this process could also occur in the isolated kidney of the cat perfused *in vitro* with blood containing dopa. The injection of 5-6 cc of the perfusate into a test cat caused a rise in blood pressure of 120 mm Hg. The amount of the pressor substance produced, presumably hydroxytyramine, was found to vary inversely with the rate of blood flow through the kidney. Since these experiments suggested that renal hypertension is due to a similar transformation of amino acids by the ischemic kidney, experiments were performed to investigate (a) whether amino acids other than those examined by Holtz could be transformed into pressor substances by renal tissue extracts and by perfused kidneys, (b) whether the enzymes responsible for this process varied with different species, and (c) whether renin, a protein found in renal extracts[4] could transform dopa into hydroxytyramine.

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[4] Osborne, T. B., and Mendel, L. B., *J. B. C.*, 1918, **34**, 131.

[1] Holtz, P., *Klin. Woch.*, 1937, **16**, 1561.

[2] Holtz, P., and Heise, R., *Arch. Exp. Path. und Pharm.*, 1939, **191**, 87.

[3] Bing, R. J., *Am. J. Physiol.*, 1941, March.

For the perfusion and incubation experiments the technic described by one of us (R.J.B.) was used.[3, 5] The perfusion fluid for the cat's kidney consisted of 120 cc of heparinized cat's blood; for the human kidney 120 cc of human blood containing 5% citrate were used. The lung was included in the perfusion circuit except in experiments on the human kidney. Cats anesthetized with nembutal (39 mg per kg body weight intraperitoneally) were used to ascertain the effect of the protein-free extracts and the kidney perfusates on the blood pressure. All samples were injected intravenously.

In 21 incubation experiments *l*-dopa,* thyroxine,† *d-l*-phenylalanine and *l*-tryptophane were incubated under nitrogen for 12-16 hours at 38°C with extracts from the renal cortex of the guinea pig, cat and man. An equal amount of the amino acids was incubated with the kidney extracts under oxygen. As seen in Table I, the anaerobic incubation of dopa with renal cortical extracts of these 3 species gave rise to a pressor substance. Both the height and duration of the response were enhanced by the previous injection of cocaine hydrochloride (6 mg per kg body weight intravenously) in the experiments on guinea pig's renal extracts. In experiments on extracts of the kidney of the cat, cocaine caused a slight decrease in the height of the blood pressure response, but usually increased its duration. The work of Holtz makes it probable that this pressor substance is identical with hydroxytyramine. The injection of 6 mg of dopa dissolved in Ringer's solution had no

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[4] Helmer, O. M., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 757.

[5] Bing, R. J., *Am. J. Physiol.*, 1941, in press.

* Hoffman-LaRoche.

† Squibb, Thyroxin Crystals. Natural form possibly converted to racemic mixture by crystallization.

TABLE I.
Incubation of Renal Extracts under Nitrogen.

| Species | Amino-acid | Mg amino-acid used | No. exp. | Cc concentrate inj.* | Rise in blood pressure* |  |
|---|---|---|---|---|---|---|
|  |  |  |  |  | Before cocaine hydrochloride | After cocaine hydrochloride |
| Guinea pig | *l*-dopa | 125 | 6 | 1 | 90 | 145 |
| ,, ,, | thyroxine | 10 | 1 | 2.5 | 0 |  |
| ,, ,, | *d*-*l*-phenylalanine | 35 | 3 | 2.3 | 100 | 35 |
| Cat | *l*-dopa | 5 | 4 | 1 | 70 | 65 |
| ,, | *d*-*l*-phenylalanine | 35 | 1 | 2 | 0 |  |
| ,, | *l*-tryptophane | 20 | 2 | 2 | 0 | 0 |
| Human | *l*-dopa | 35 | 1 | 2 | 110 |  |
| ,, | ,, | 5 | 1 | 2 | 80 |  |

*Typical experiment.

effect on the blood pressure. A pressor substance also originated during the anaerobic incubation of phenylalanine with renal cortical extracts of the guinea pig. Its pressor effect was decreased after the previous injection of cocaine. During the anaerobic incubation of extracts of phenylalanine with cat's kidneys, however, no pressor substance was formed. Protein-free extracts obtained from guinea pig's kidneys incubated with thyroxine and of cat's kidneys with tryptophane had no effect on the blood pressure of the test cat.

Holtz showed that an enzymatic de-amination of hydroxytyramine occurred when the incubation of dopa and renal extracts was carried out under oxygen.[2] Extracts of the cat's kidney, however, incubated under oxygen with 5 mg of dopa usually gave a pressor response, but the rise in blood pressure was always smaller than that caused by the anaerobically incubated extract.

In the perfusion experiments, cat and human kidneys were perfused with reduced blood flow. After removing a sample of blood to serve as a control, 240 mg of dopa were added to the perfusate and perfusion was continued for 2 hours. The effect of the injection of 3.5-5 cc of the perfused plasma on the blood pressure of the test cat was compared with that produced by an equal amount of control plasma. The control samples had no effect. As shown in Table II, dopa was converted into a pressor substance, presumably hydroxytyramine, by both the perfused human and cat's kidneys. No pressor substance was detected in plasma perfused through the cat's kidney with phenylalanine.

Since there was a possibility that the enzyme responsible for the decarboxylation was renin, 1 cc of renin solution‡ was incubated in

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‡ Kindly supplied by Dr. I. H. Page of the Lilly Laboratory, Indianapolis.

TABLE II.
Perfusion Experiments.

| Species | Amino-acid | Reduction in blood flow (cc/min)* | Cc plasma inj.† | Rise in blood pressure† (mm Hg) | |
|---|---|---|---|---|---|
| | | | | Before cocaine hydro-chloride | After cocaine hydro-chloride |
| Cat | *l*-dopa | 12 | 5 | 120 | 140 |
| " | " | 5 | 5 | 30 | 40 |
| " | *d-l*-phenylalanine | 5 | 4 | 0 | |
| Human | *l*-dopa | 20 | 3.5 | 157 | |
| " ‡ | " | 20 | 4 | 30 | 35 |

*Average value before reduction of flow in the cat was 17.5 cc per min and in the human 40 cc per min.

†Typical experiment.

‡Kidney obtained 12 hours postmortem. Destruction of the decarboxylating enzyme by autolysis probably explains the small yield of pressor substance.

one experiment for 10 minutes, in another for 2 hours with 35 mg of dopa in 50 cc of phosphate buffer under nitrogen. After incubation, the renin was destroyed by heating. Neither preparation gave a pressor response when injected into a test cat. It can be concluded, therefore, that renin was unable to convert dopa into hydroxytyramine. Incubation of 0.5 cc of renin and 25 mg of dopa in 5 cc of Ringer's solution with 11 cc of serum, which contains renin activator,[6] also failed to produce hydroxytyramine.

*Discussion.* It is shown that during anaerobic incubation of the extracts of guinea pig kidneys with either dopa or phenylalanine, substances originate which raise the blood pressure of the anesthetized cat. It can be assumed on the basis of the work by Holtz, that these substances are amines produced by the decarboxylation of dopa or phenylalanine. Extracts prepared after the incubation of renal cortical extracts of the cat with tryptophane and of the guinea pig with thyroxine have no effect on the blood pressure of the cat. This observation confirms the conclusion of Holtz that the decarboxylases are specific for certain amino acids.[7] Both anaerobic incubation and perfusion of human kidneys with dopa give rise to a pressor substance, demonstrating that the human kidney contains a dopa decarboxylase.

In experiments in which extracts of cat's kidneys are anaerobically incubated with phenylalanine no pressor substance is formed, indicating that, in contrast to the guinea pig's kidney, that of the cat probably does not contain a phenylalanine decarboxylase. Experiments in which the cat's kidney is perfused with blood containing phenylalanine confirm these results. These experiments demonstrate that the decarboxylating enzyme responsible for the transformation of phenylalanine into its corresponding amine is species specific.

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[6] Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 214.

[7] Holtz, P., Credner, K., and Walter, H., *Z. f. physiol. Chem.*, 1939, **262**, 111.

*Summary.* The decarboxylating enzymes contained in the kidney are specific for certain amino acids and vary with the species.

We express our sincere appreciation to the Department of Urology of the Presbyterian Hospital, for help in providing us with the human material.

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## 11989

## Quantitative Spectroscopic Analysis of Stem "Tracheal" Fluids for Inorganic Constituents.*

PHILIP HAMM, R. B. HARVEY AND ELMER S. MILLER.
*From the Departments of Botany and Plant Pathology, University of Minnesota.*

Many workers have reported quantitative spectrographic methods for the determination of major and trace elements necessary for plant growth. The major plant nutrition studies by spectrographic analysis have concerned either leaf tissue analysis or plant ash analysis Heretofore, the general practice consisted of volatilizing a homogenized ash in a 220 volt d-c arc with a current of 9-12 amperes. Estimations of the amounts of the constituents present have usually been carried out either by employing standard powders according to Nitchie[1] for comparison purposes, or by using suitable internal standards and determining the ratios of certain emission lines of the unknown to the suitable emission spectra of an internal standard as outlined by Gerlach.[2] Studies of ashed leaf tissue have furnished data concerning the number and respective amounts of the soil elements that are taken up by the plant. However, because only a portion of each element present in a plant ash is available in a mobile form, it has been difficult to interpret the significance of these analyses. Recently, Duffendack and Wolffe[3] and Hamm, *et al.*,[4] have presented evidence that both accuracy and sensitivity may be increased by employing an a-c arc across the graphite electrodes. This additional sensitivity has extended the spectrographic method to such low concentrations (10 parts per billion) as found in certain tissue fluids.

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* Aided by research grants from the Graduate School, University of Minnesota, Minneapolis, Minnesota. Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official No. 65-1-71-140, Sub-project No. 331.

The authors wish to express their sincere thanks to Dr. R. E. Oltman for assistance in certain studies on electrode impurities.

[1] Nitchie, C. C., *Ind. and Eng. Chem.* Analyt. Ed., 1929, **1**, 1.

[2] Gerlach and Schweitzer, *Foundations and Methods of Chemical Analysis by the Emission Spectrum.*

The purpose of this paper is to present in detail a rapid and direct spectroscopic method for the quantitative analysis of the inorganic constituents of plant stem "tracheal" fluids, which permits the determination of what elements are absorbed and are available at any phase of plant growth.

*Experimental.* Four to 12 plant stems are collected and cut into pieces 4 inches long. Usually, it is necessary to store the stems

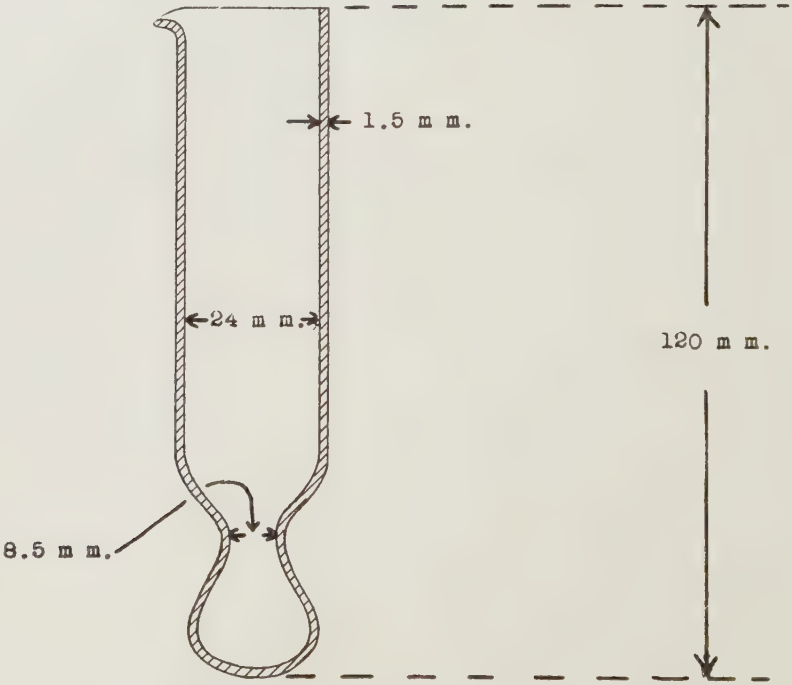

FIG. 1.
Centrifuge tube for collecting tracheal fluids.

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[3] Duffendack, O., and Thompson, *Proc. Am. Soc. Testing Materials,* **1936, 36,** 310.

[4] Hamm, Philip, *et al.* (In press.)

24 to 72 hours to permit transportation to the laboratory. Under such conditions, immediately upon collection in the field, the stems are dipped in a low melting paraffin to minimize desiccation while samples are in transit and storage. In the laboratory after the ends of the stem are cut off and the stems brushed free of dirt, the sample is placed in specially designed centrifuge tubes (Fig. 1). The stems are centrifuged at 1500 r.p.m. for 15 minutes. The exact centrifugal force required is determined somewhat by the species and the time of collection. Six 4-inch potato stems will yield 0.10 to 0.6 cc tracheal fluid. Should several centrifuge tubes be necessary for the collection of the sample, all portions are combined to give a composite example.

*Preparation of Graphite Electrodes.* For these studies graphite electrodes "Spectroscopic Carbons" (National Carbon Company, Cleveland) 0.25 inch in diameter are employed. The ends of the graphite electrodes are rendered flat by touching the end against a motor-driven grinder. The electrodes are pretreated by 2 drops of Skelly-solve containing 1% paraffin. Aliquot portions, 0.075 ml, of "tracheal" fluid are deposited on the end of the graphite electrodes in quadruplicates. After the fluid is evaporated to dryness under an infra-red lamp, the internal standard, in this case lead acetate, is deposited on the end of the electrode. It is preferable to add the internal standard separately to avoid precipitation of certain fluid constituents. The transit holder with graphite electrodes is placed under the infra-red lamp until the sample is *dry*. The entire holder is then placed in a desiccator until the spectrographic analyses are made. The piece of graphite containing the sample forms the lower electrode 1 mm below the upper piece of graphite. The sample is excited in an a-c arc at 3000 volts and 2.5 amperes and 60-second exposures are employed. Any fluctuation in the arc is reflected by the density of the emission lines of the internal standard. The plates, either Cramer contrast or Eastman 103-O, are developed to a gamma of about 0.6 for those elements in the lowest concentration. The density progression with respect to concentration is extended by including a step sector in the optical train. The absolute concentration of any constituent is determined by constructing a working curve, by plotting the log of the ratio of the density of a certain line to that of a suitable emission line of the internal standard against concentration of the element on the other axis. These working curves are constructed by obtaining a series of spectrograms of an artificial standard at different dilutions through

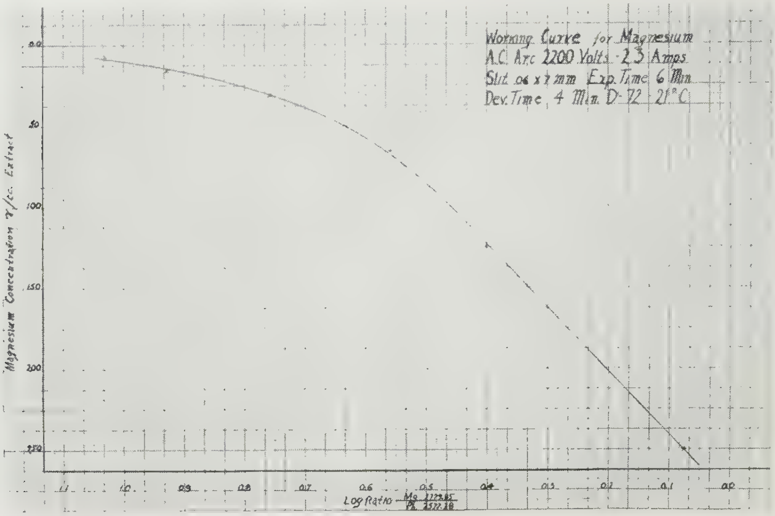

FIG. 2.
A working curve for quantitative analyses of magnesium by emission spectra.

a concentration range corresponding to the range found in the tracheal fluid investigated (Fig. 2).

*Electrode Impurities.* Because the concentrations of inorganic constituents in the tracheal fluid are so much lower than that in the leaf tissue, particular precaution must be observed insofar as electrode impurities are concerned. The inexpensive grade of "Spectroscopic Carbons" distributed by the National Carbon Co., Inc., revealed on spectroscopic examination the impurities listed in Table I.

The highest quality of graphite electrodes contain minute quantities (in concentrations less than 0.01 p.p.m.) of copper, calcium, iron and silicon. Because of these impurities a "blank" correction at equivalent exposures must be made in using the working curves.

Typical analyses of potato "tracheal" fluid are presented in Table II.

TABLE I.
Impurities in Graphite Electrodes as Determined by an A-C Arc.

| Element | p.p.m. |
|---|---|
| Boron | 0.01 |
| Magnesium | 0.10 |
| Manganese | 0.01 |
| Calcium | 0.01 |
| Copper | 0.02 |
| Silicon | appreciable |
| Iron | ,, |
| Aluminum | trace |

TABLE II.
Quantitative Analysis of Inorganic Constituents in Potato Stem "Tracheal" Fluid.

| Element<br>Treatment | B<br>p.p.m. | P<br>p.p.m. | Mn<br>p.p.m. | Mg<br>p.p.m. | Cu<br>p.p.m. | Ca<br>p.p.m. |
|---|---|---|---|---|---|---|
| B | 0.80 | 27.6 | .40 | 345 | .65 | 110 |
| Zn | 1.80 | 18.0 | .23 | 348 | .90 | 136 |
| Mn | 1.20 | 18.0 | .38 | 366 | — | 125 |
| K | 1.10 | 18.0 | .44 | 363 | .55 | 183 |
| Mix | 1.20 | 17.0 | .52 | 399 | — | 183 |
| Control | 0.40 | 10.3 | .22 | 294 | — | 62 |

*Summary.* (1) A rapid and reliable method for quantitatively analyzing "tracheal" fluid by emission spectroscopy has been described in detail. (2) Evidence has been presented showing that a correction should be made for electrode impurities. (3) Typical analyses of potato tracheal fluid for B, P, Mn, Mg, Cu, and Ca are presented.

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## 11990

## Etioallocholanol-3($\beta$)-17-One (Isoandrosterone) as a Metabolite of Testosterone in the Human Male.*

RALPH I. DORFMAN.

*From the Laboratory of Physiological Chemistry and the Adolescence Study Unit, Yale University School of Medicine, New Haven, Conn.*

Continuing the study of the metabolic fate of testosterone in the human male, we have now been successful in demonstrating that etioallocholanol-3($\beta$)-17-one (isoandrosterone) is a possible metabolite of testosterone. Previous communications have established androsterone and etiocholanol-3($\alpha$)-17-one as metabolic products of testosterone in the human male.[1, 2, 3] When testosterone was administered to the adult male guinea pig, etioallocholanol-3($\beta$)-17-one was recovered in the urine.[4]

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* This work was aided by grants from the Rockefeller Foundation, the Fluid Research Fund of Yale University School of Medicine, and the International Cancer Research Foundation. The latter grant was made to Dr. James B. Hamilton of the Department of Anatomy.

[1] Callow, N. H., *Biochem. J.*, 1939, **33**, 559.

[2] Dorfman, R. I., Cook, J. W., and Hamilton, J. B., *J. Biol. Chem.*, 1939, **130**, 285.

[3] Dorfman, R. I., PROC. SOC. EXP. BIOL. AND MED., 1930, **45**, 739.

[4] Dorfman, R. I., and Fish, W. R., *J. Biol. Chem.*, 1940, **135**, 349.

The presence of etioallocholanol-3(β)-17-one has been established in the urine of a patient suffering from adrenal hyperplasia.[5, 7] Recently, this substance has been isolated from the urine of normal women and also from the urines of both men and women suffering from cancer.[6]

The urine was collected from a 30-year-old hypogonadal male during the time the individual was being injected intramuscularly with 30 mg of testosterone propionate† per day. During this period the daily excretion of androgenic substances exceeded 75 International Units (I.U.) per day compared to the value of 14 I.U. per day in the absence of any treatment. A total of 12 days of urine during this treatment was collected.

The urine was extracted in the usual manner and the neutral compounds separated into the ketonic and nonketonic fractions by means of the Girard-Sandulesco reagent.[2]

The neutral ketonic compounds were fractionated by means of chromatographic analysis using aluminum oxide (Brockmann) as the absorbent and carbon tetrachloride as the solvent. That fraction which was eluted with carbon tetrachloride containing 0.1% ethanol yielded a crystalline residue on evaporation of the solvent. This crystalline residue was dissolved in 4 cc of hot 90% ethanol, and 170 mg of digitonin in 8 cc of hot 90% ethanol was added. The precipitate that formed after 2 hours standing was collected and washed with cold 90% ethanol and ether. After decomposing the digitonide with dry pyridine and ether, a crystalline compound was isolated which melted at 150-154°C. After 3 recrystallizations from aqueous methanol, 35 mg of a crystalline substance melting at 168-170°C was isolated. When this substance was mixed with an equal amount of an authentic sample of etioallocholanol-3(β)-17-one, melting point 170°C, the melting point was 169-170°C. The benzoate of the compound melted at 212-214°C while the acetate melted at 112°C. The melting points of respective mixtures of the benzoate and acetate with authentic samples were 213-215° and 115°.

Since the amount of etioallocholanol-3(β)-17-one isolated amounted to 2.9 mg per day, it seems unlikely that this substance was produced by this individual's own gonads or adrenal cortex. In earlier experiments we were unable to detect the presence of

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[5] Butler, G. C., and Marrian, G. F., *J. Biol. Chem.*, 1938, **124**, 237.

[6] Pearlman, W. H., *J. Biol. Chem.*, 1940, **136**, 807.

[7] Marrian, G. F., and Butler, G. C., *Nature*, 1938, **142**, 400.

†Testosterone propionate was furnished by Ciba Pharmaceutical Products, Inc., under the trade name, Perandren.

etioallocholanol-3($\beta$)-17-one in the urine of this patient collected during an 8-day noninjection period.

The isolation of etioallocholanol-3($\beta$)-17-one after the administration of testosterone to the human seems to demonstrate the third of a possible 4 androsterone isomers that may arise by the reduction of the $\alpha,\beta$ unsaturated ketone ring A of testosterone. The isolation of the other 2 isomers, that is, androsterone and etiocholanol-3($\alpha$)-17-one, have been adequately described previously.[1, 2, 3] Only one isomer now remains to be isolated, this one being etiocholanol-3($\beta$)-17-one. Attempts are being made to demonstrate this substance as a metabolite of testosterone.

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### 11991

## Cure of Signs of Egg White Disease by Corn Oil Fatty Acids and Vitamin $B_6$.

Eaton M. MacKay and Richard H. Barnes.
*From the Scripps Metabolic Clinic, La Jolla, California.*

Gyorgy, *et al.*,[1] believe that the various nutritional dermatoses of the rat are clinically quite different. However, there appear to be some similarities between "egg white" dermatitis[2, 3] and the signs of the fat deficiency disease described by Burr and Burr[4] which is due to the absence of certain essential fatty acids from the diet. The influence upon "egg white" disease of corn oil which is rich in one of these—linoleic acid—has been determined. A typical experiment is described. This type of experiment has been repeated twice with larger groups of animals in very nearly the same manner and with identical results.

A diet composed of casein 50, sucrose 20, Osborne and Mendel[5] salt mixture 5, brewer's yeast (Anheuser-Busch strain G) 5, cod liver oil (tested) 5, and Crisco 15 allows normal growth, maintenance and reproduction in the albino rat without any evidence of alopecia or skin pathology. When the casein in this diet is replaced by commercial dried egg white the characteristic dermatitis of "egg white disease"[3] is produced. This egg white diet was fed to Groups C and D (3 female rats in each group) of this study. The diets fed to groups A and B were similar except for the replacement of 10% of the Crisco with a commercial corn oil (Mazola, a corn oil from which some of the saturated fatty acids have been removed) as a source of linoleic acid. The results were very striking. Almost from the start (when the rats were 40 days old) of the feeding of the egg white diets the rats receiving corn oil (Groups A and B) gained weight better (Fig. 1) than the others (Groups C and D). After 60 days upon the diets, egg white acrodynia was well developed in the rats receiving no corn oil (Table I, 100 days old observations) while there were only questionable spectacled eyes in the corn oil groups.

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[1] Gyorgy, P., Sullivan, M., and Karsner, H. T., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 313.

[2] Boas, M. A., *Biochem. J.*, 1927, **21**, 712.

[3] Parsons, H. T., *J. Biol. Chem.*, 1931, **90**, 351.

[4] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

[5] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, **34**, 131.

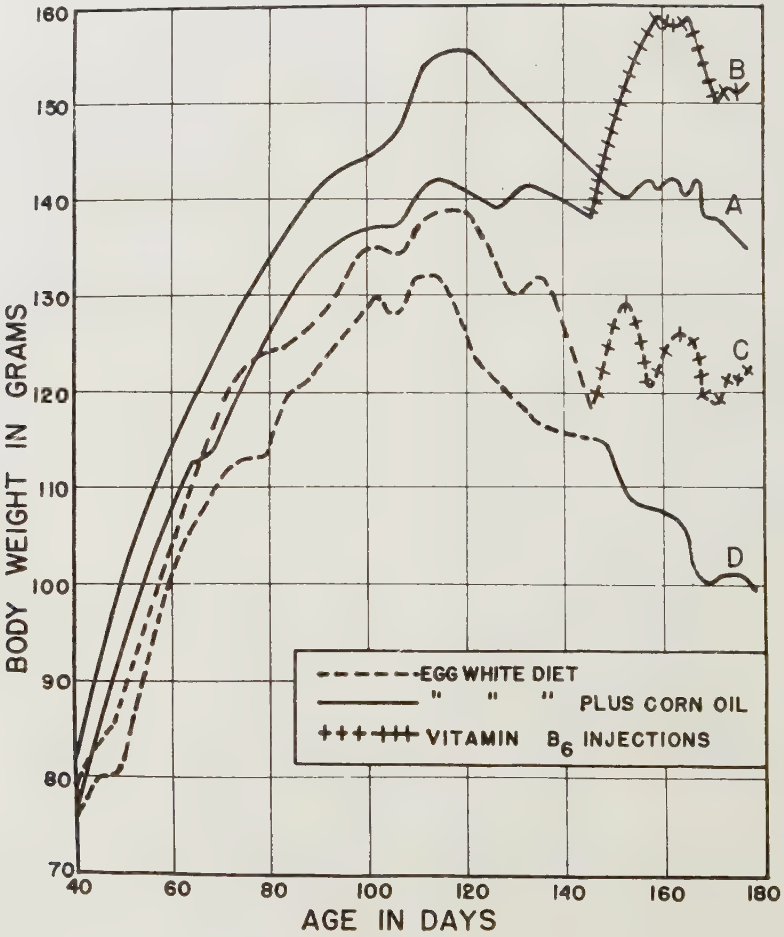

TABLE I.
Influence of Corn Oil and Vitamin $B_6$ on the Signs of Egg White Acrodynia in the Albino Rat.

| Group | Age in days | Treatment |  | Eyes |  | Skin |  |  |
|---|---|---|---|---|---|---|---|---|
|  |  | Corn oil | Vitamin $B_6$ | Spectacled | Closed | Erythema | Scaliness | Alopecia |
| A | 100 | + | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 100 | + | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 100 | 0 | 0 | + | + | ++ | + | + |
| D | 100 | 0 | 0 | + | + | ++ | + | + |
| A | 146 | + | 0 | + | 0 | + | + | + |
| B | 146 | + | 0 | + | 0 | + | + | + |
| C | 146 | 0 | 0 | ++ | + | +++ | +++ | +++ |
| D | 146 | 0 | 0 | ++ | + | +++ | +++ | +++ |
| A | 160 | + | 0 | + | + | ++ | ++ | ++ |
| B | 160 | + | + | 0 | 0 | 0 | 0 | ? |
| C | 160 | 0 | + | 0 | 0 | ++ | ++ | ++ |
| D | 160 | + | 0 | + | + | ++++ | +++ | +++ |
| A | 177 | + | 0 | + | + | ++ | ++ | ++ |
| B | 177 | + | + | 0 | 0 | 0 | 0 | ? |
| C | 177 | 0 | + | 0 | 0 | + | + | ++ |
| D | 177 | + | 0 | + | + | +++ | +++ | +++ |

At 146 days of age, 106 days after the diet was first administered, the signs of egg white disease were quite marked (Table I) in the groups (C and D) receiving no corn oil and they were losing weight at a rapid rate (Fig. 1). The rats receiving corn oil (Groups A and B) were in much better condition but had lost some hair and there was definite erythema of the belly and forepaws with some scaliness of the skin elsewhere. The sticky secretion which closed the eyes of the rats in the other groups all of the time led to sticking of the lids a fair portion of the time in the rats getting corn oil. In brief, the presence of 10% corn oil in the diet had not prevented the occurrence of the egg white disease but had made it much less severe and the signs of the ailment developed much more slowly than in rats which were not given corn oil.

Birch and Gyorgy[6] suggested a sparing action of linoleic acid on vitamin $B_6$ which in its own deficiency leads to the development of an acrodynia in the rat. This synergism between vitamin $B_6$ and linoleic acid has been further described by Birch[7] and Salmon.[8] In order to determine the relation of vitamin $B_6$ to egg white disease the rats in 2 of our groups (B and C) were each given 0.2 mg daily of vitamin $B_6$ by subcutaneous injection after they had been on the special diets 106 days. In Group C, which had no corn oil, this stopped the loss of weight and there was even an evanescent gain in weight. There was a definite improvement in the dermatitis and some of the lost hair was regained. The other group (D), which had not been given corn oil, was changed to the corn oil diet at 146 days of age but the rats continued to fail and lose weight. The very severe dermatitis in this group did not improve and 2 of the 3 rats died just before the experiment was terminated.

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[6] Birch, T. W., and Gyorgy, P., *Biochem. J.*, 1936, **30**, 304.

[7] Birch, T. W., *J. Biol. Chem.*, 1938, **124**, 775.

[8] Salmon, W. D., *J. Biol. Chem.*, 1940, **133**, lxxxiii.

Group A, which had been given corn oil in the diet from the start of egg white feeding, continued to lose weight at a slow rate while the condition of the skin became steadily worse and reached a stage (Table I) similar to that reached by the non-corn oil fed rats at a much earlier period. The corn oil fed group (B) which was also given vitamin $B_6$ showed a remarkable improvement in their condition. They gained weight rapidly and in 14 days had lost all evidence of acrodynia which they had begun to exhibit earlier. The lost hair was rapidly replaced and a questionable alopecia is indicated for this group at the end of the experiment only because the hair growth was not quite as thick as with rats of the same age raised entirely on the stock diet. The injection of vitamin $B_6$ cured all of those signs of egg white disease which were not prevented by the inclusion of corn oil in the diet.

*Discussion.* A constituent of various foodstuffs which will overcome egg white injury has long been recognized.[2, 9, 10] Gyorgy and coworkers[11, 12] refer to this factor as vitamin H and du Vigneaud, *et al.,*[13] have shown the identity of Gyorgy's vitamin H with biotin. Our experiments raise the possibility that corn oil and vitamin $B_6$ may replace or enhance biotin activity. Schneider, *et al.,*[14] find that non-egg white acrodynia may be cured by either the "essential fatty acids" alone or vitamin $B_6$ plus an accessory factor. If this factor was biotin our results might be explained on this basis. The question of nutritional dermatoses is without doubt a complex one but an adequate supply of linoleic acid in the diet would appear to be desirable in studying any type of acrodynia.

*Summary.* Egg white injury or acrodynia in the albino rat, which occurs when commercial dried egg white is included in the diet of

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[9] Lease, J. G., and Parsons, H. T., *Biochem. J.*, 1934, **28**, 2109.

[10] Gyorgy, P., *J. Biol. Chem.*, 1939, **131**, 733.

[11] Gyorgy, P., Kuhn, R., and Lederer, E., *J. Biol. Chem.*, 1939, **131**, 745.

[12] Birch, T. W., and Gyorgy, P., *J. Biol. Chem.*, 1939, **131**, 761.

[13] du Vigneaud, V., Melville, D. B., Gyorgy, P., and Rose, C. S., *Science*, 1940, **92**, 62.

[14] Schneider, H., Steenbock, H., and Platz, B. R., *J. Biol. Chem.*, 1940, **132**, 539.

this animal in a substantial amount develops much more slowly if 10% of the fat in the diet is in the form of corn oil instead of Crisco. If vitamin $B_6$ is injected into corn oil fed rats the signs and symptoms of the egg white acrodynia almost entirely disappear.

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### 11992

## Studies on Identity of Prothrombin.

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Since Schmidt's[1] work in 1872, there have been many attempts to isolate and identify thrombin and its precursor, prothrombin. Chief among these have been those of Howell,[2] Eagle,[3] Mellanby,[4, 5] Seegers,[6] and Astrup and Darling.[7] Most of these workers have believed that prothrombin is a globulin. Mellanby, Seegers, and Astrup and Darling have probably isolated thrombin in its purest form, and Mellanby has investigated its chemical composition as well as its physiological properties. More recently, Astrup, *et al.*, have, on the basis of precipitation studies with ammonium sulphate, demonstrated that the precipitation pattern of thrombin was that of albumin.

In the course of plasma protein studies in multiple sclerosis,[8] using Butler's[9] method of phosphate buffer precipitation and the Tiselius electrophoresis technic, opportunity was presented to obtain additional data on the identity of prothrombin. Our aim was to show with what fraction of plasma proteins prothrombin precipitated, and how it migrated when subjected to electrophoresis by Tiselius's technic

Oxalated human blood plasma was mixed with Butler's phosphate buffer in equal parts beginning at a concentration of 1.3 molar phosphate of resultant mixture, and increasing with an increment of 0.1 molar through 2.5 molar. The mixtures were allowed to stand several hours at room temperature, and filtered through Whatman No. 50 paper. Each precipitate and filtrate was saved. The precipitates were washed with buffer of molar strength equal to that used in the precipitation and washings discarded. The washed precipitates were dissolved in distilled water and all (filtrates and precipitates) were dialyzed for 12 hours in Viscose casings in running tap water to remove most of the phosphate. These resulting solutions were made up to equal volume,[5] and after activation with 3% rabbit brain extract and calcium they were tested for their thrombin content by the speed with which they clotted fibrinogen solution.

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[1] Schmidt, Alexander, *Pfluger's Arch.*, 1872, **6**, 445.

[2] Howell, William H., *Am. J. Physiol.*, 1914, **35**, 474.

[3] Eagle, Harry, *J. Gen. Physiol.*, 1935, **18**, 531.

[4] Mellanby, J., *Proc. Roy. Soc.*, London, Ser. B, 1931, **107**, 271.

[5] *Ibid.*, 1933, **113**, 93.

[6] Seegers, W. H., Smith, H. P., Warner, E. D., Brinkhous, K. M., *J. Biol. Chem.*, 1938, **123**, 751.

[7] Astrup, Tage, and Darling, Sven, *J. Biol. Chem.*, 1940, **133**, 761.

[8] Putnam, Tracy J., to be published.

[9] Butler, Allan M., and Montgomery, Hugh, *J. Biol. Chem.*, 1932, **99**, 173.

From the results (Chart 1) it is seen that prothrombin precipitates

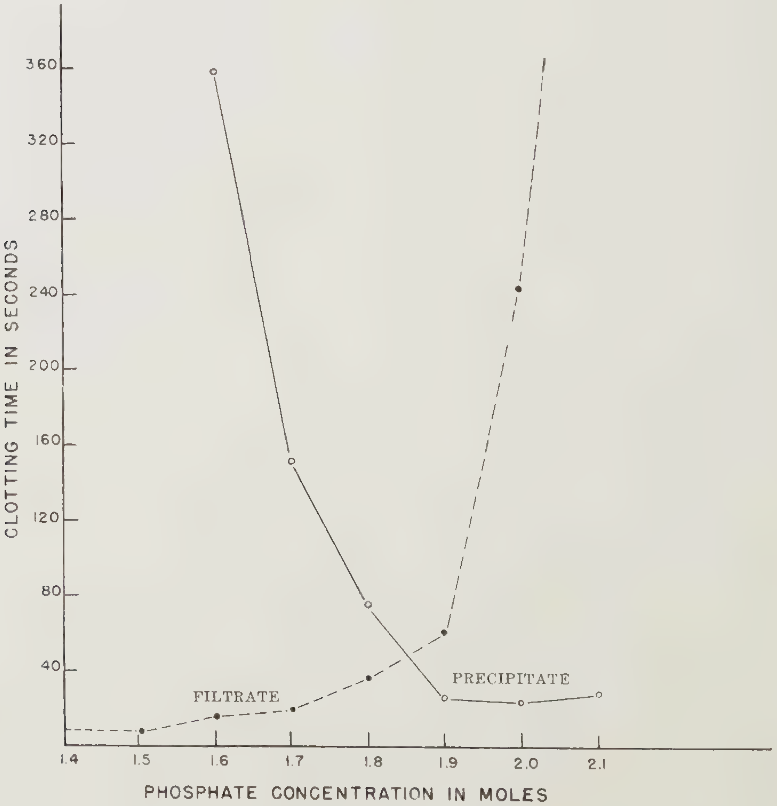

Chart 1.

Clotting time of fibrinogen by both precipitate and filtrate of plasma proteins plotted against molar strength of phosphate at which the division was made.

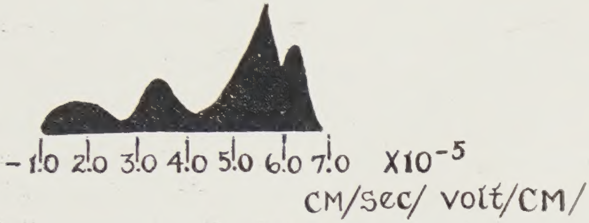

CHART 2.

Electrophoretic pattern of plasma proteins of high prothrombin content. Mobilities are given by position of peaks and relative concentration by areas under them.

between 1.7 and 2.1 molar phosphate which corresponds to the upper ranges of globulin solubility.

A similar preparation of plasma proteins precipitating between 1.7 and 2.0 molar phosphate was made, such that the final volume would be about 1/10 that of the original plasma, dialyzed 6 hours in running tap water and overnight in distilled water containing a small amount of potassium oxalate (about 0.01%) to precipitate any calcium which might be present. A considerable quantity of inert material precipitated during dialysis, and was spun out. One cc of the resulting solution, after activation, clotted 1.0 cc of fibrinogen solution in 3 seconds. Electrophoretic pattern (in 0.025 M barbiturate buffer at pH 7.76) of the above is found in Chart 2. The mobility of the chief peak in $cm^2\ volt^{-1}\ sec^{-1}$ was $5.8\ 10^{-5}$ which most closely approximates that of alpha globulin.* It is to be observed, however, that the electrophoretic pattern appears to display all of the globulins and some albumin although the protein had been separated over a narrow range of salt concentration. This demonstrates again the inadequacy of salting methods for separating proteins.

Untreated oxalated plasmas from control healthy individuals and multiple sclerotic patients were subjected to electrophoresis in 0.025 M barbiturate buffer at pH 7.8, and the various components separated out in two parts. Each part was tested for prothrombin content (as before) with the following results:†

| Prothrombin absent | Prothrombin present |
|---|---|
| 1. gamma | phi alpha beta albumin |
| 2. gamma phi beta | alpha; albumin |
| 3. gamma phi beta alpha | albumin |

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* The mobilities of the peaks of the protein preparation differ markedly from that of whole plasma.

† It is interesting to note that the globulin fractions obtained by precipitation did not require with phosphate buffer the addition of thromboplastin to activate the prothrombin (though the reaction was accelerated by it). The Tiselius albumen fraction, however, could not be activated by calcium alone, and required the presence of thromboplastic substance.

Experiment 3 was repeated 3 times and on all occasions, the prothrombin was found to migrate solely with the albumin.

*Summary.* 1. With Butler's phosphate buffer as a salting solution, prothrombin precipitates between 1.7 and 2.0 molar strength, which places it in the upper globulin fraction. 2. The bulk of proteins of solubility similar to prothrombin migrates electrophoretically as alpha globulin. 3. On the electrophoresis of whole plasma, prothrombin migrates with the albumin fraction.

# ERRATUM

Secretion of Inulin by Kidney of Crayfish, 1940, p. 873, by N. S. Rustum Maluf, legend, Fig. 2 should read: "Inulin-clearance in cc per hour (ordinate) as a function of the U/P ratio of inulin (abscissa). Each point is for a separate crayfish; the same individuals as in Fig. 1."